Tesis doctoral

Análisis de los ciclos de nutrientes en bosques de *Quercus* afectados por decaimiento: conectando cambios en la comunidad con el funcionamiento del ecosistema

Consejo Superior de Investigaciones Científicas
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ANALYSES OF NUTRIENT CYCLES IN OAK FORESTS AFFECTED BY DECLINE: LINKING COMMUNITY CHANGES WITH ECOSYSTEM FUNCTIONING

Memoria presentada por **José Manuel Ávila Castuera** para optar al **título de Doctor** con mención internacional por la Universidad Pablo de Olavide

Esta memoria ha sido realizada bajo la dirección de: Dra. Lorena Gómez-Aparicio y Dr. Antonio Gallardo Correa

Ldo. José Manuel Ávila Castuera
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Dra. Lorena Gómez Aparicio, Investigadora del Instituto de Recursos Naturales y Agrobiología y **Dr. Antonio Gallardo Correa**, Catedrático de Ecología de la Universidad Pablo de Olavide

CERTIFICAN

Que los trabajos de investigación desarrollados en la memoria de tesis doctoral titulada: **"Análisis de los ciclos de nutrientes en bosques de *Quercus* afectados por decaimiento: conectando cambios en la comunidad con el funcionamiento del ecosistema"** han sido realizados bajo nuestra dirección en el Instituto de Recursos Naturales y Agrobiología y son aptos para ser presentados por el Ldo. José Manuel Ávila Castuera ante el Tribunal que en su día se designe para aspirar al Grado de Doctor con Mención Internacional por la Universidad Pablo de Olavide.

Y para que así conste, y en cumplimiento de las disposiciones legales vigentes extendemos el presente certificado a 25 de enero de 2019.

Dra. Lorena Gómez-Aparicio

Dr. Antonio Gallardo Correa

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Los trabajos realizados en esta tesis doctoral se realizaron en el Departamento de Biogeoquímica, Ecología vegetal y Microbiana del Instituto de Recursos Naturales y Agrobiología de Sevilla (Consejo Superior de Investigaciones Científicas) y en el Departamento de Sistemas Físicos, Químicos y Naturales (Área de Ecología) de la Universidad Pablo de Olavide.

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A mi gran familia

A mi nena

Great works are performed not by strength,
but by perseverance

Los grandes trabajos no son hechos con la fuerza,
sino con la perseverancia

Samuel Johnson

RESUMEN GENERAL

En las últimas décadas se ha detectado un incremento en las tasas de mortalidad en bosques a escala mundial, cuyas principales causas han sido atribuidas a fenómenos asociados al cambio global como el cambio climático y las plagas de insectos y patógenos invasores. El proceso de defoliación y muerte asociado a estos fenómenos podrían tener grandes impactos directos e indirectos sobre el funcionamiento del ecosistema. Sin embargo, nuestro conocimiento actual de las consecuencias del decaimiento es aún limitada y fuertemente sesgada a determinados eventos de mortalidad, como los asociados a incrementos de temperatura o las plagas de insectos. El objetivo general de esta tesis doctoral es analizar las consecuencias para el funcionamiento del ecosistema, y particularmente para los principales ciclos biogeoquímicos (carbono, nitrógeno y fósforo), de cambios en la estructura y composición de bosques mixtos del sur de la península ibérica afectados por el decaimiento de su especie dominante, *Quercus suber*, debido al patógeno invasor *Phytophthora cinnamomi*. Los trabajos experimentales de esta tesis se realizaron en seis parcelas situadas en el parque natural Los Alcornocales (Cádiz), tres de ellas localizadas en bosques abiertos dominados por *Q. suber* y *Olea europaea* var. *sylvestris* y otras tres localizadas en bosques cerrados de *Q. suber* y *Q. canariensis*.

En el **capítulo 2** de esta tesis doctoral se analizó la capacidad de los individuos de *Q. suber* de ajustar su fisiología (eficiencia del uso del agua y crecimiento secundario) ante el fenómeno de decaimiento asociado al patógeno *P. cinnamomi* a diferentes escalas espaciales: individual, local y regional. Los resultados de este trabajo mostraron cómo árboles sometidos a factores edáficos limitantes y a una alta abundancia de *P. cinnamomi* fueron capaces de incrementar su eficiencia del uso del agua a escala local. Sin embargo, esta capacidad es limitada y no es suficiente para que los árboles superen el estrés fisiológico asociado al decaimiento inducido por patógenos, de tal forma que árboles sanos y defoliados no mostraron diferencias en la eficiencia del uso del agua o el crecimiento secundario de sus ramas.

La elevada vulnerabilidad de *Q. suber* al patógeno invasor podría ser responsable de la elevada mortalidad que sufren los individuos de esta especie. Una vez los árboles comienzan el proceso de defoliación y muerte se producen alteraciones en las relaciones planta-suelo que pueden desencadenar importantes consecuencias para el funcionamiento del ecosistema. En los capítulos 3 y 4 se utilizaron modelos de vecindad espacialmente

explícitos para poder estimar el efecto per cápita de los árboles de diferentes especies y estado de salud sobre variables clave de los ciclos del carbono, nitrógeno y fósforo y las comunidades microbianas. Estos trabajos se realizaron cubriendo una extensa escala espacial (seis sitios de estudio pertenecientes a dos tipos de bosques) y temporal (intra e interanual), lo que ha permitido obtener unas aportaciones muy valiosas hacia el conocimiento de las consecuencias de la mortalidad arbórea asociada a patógenos en la biología del suelo. En el **capítulo 3** se muestra la fuerte relación espacial detectada entre la distribución y el estado de salud de árboles y la tasa de respiración del suelo y la disponibilidad de nutrientes. El decaimiento de *Q. suber* provocó un descenso en las tasas de respiración del suelo y la disponibilidad de fósforo, mientras que el efecto sobre la disponibilidad de nitrógeno varió entre tipos de bosque. Mientras, en el **capítulo 4** se muestra cómo el decaimiento de *Q. suber* provocó cambios en variables clave del ciclo del carbono y nitrógeno, aunque el signo y magnitud de estos cambios varió en función de textura del suelo local. Sin embargo, no se detectó ningún efecto del decaimiento de *Q. suber* ni en la diversidad funcional ni en la biomasa de la comunidad microbiana, aunque sí se observó un descenso en la respiración microbiana en el suelo. Los resultados de ambos capítulos mostraron además que existen diferencias importantes en la huella de *Q. suber* y las especies arbóreas coexistentes sobre los ciclos de carbono, nitrógeno y fósforo, lo que conllevaría importantes alteraciones de los ciclos biogeoquímicos a largo plazo si ocurriera una sustitución de *Q. suber* por otras especies no afectadas por decaimiento a lo largo del proceso de sucesión secundaria.

En el **capítulo 5** se analizaron cómo múltiples factores de cambio global afectan simultáneamente al funcionamiento del suelo en bosques mediterráneos. Concretamente, se estudiaron los efectos interactivos de la mortalidad de *Q. suber* asociada a *P. cinnamomi*, el calentamiento y la sequía sobre la respiración del suelo y la disponibilidad de amonio, nitrato y fosfato. En este trabajo se observó cómo la mortalidad inducida por *P. cinnamomi* y el futuro calentamiento y sequía podrían interaccionar para alterar simultáneamente los ciclos biogeoquímicos en suelos de bosques de *Q. suber*. Además, este trabajo demostró que tanto la respiración del suelo como la disponibilidad de nutrientes podrían ser afectados indirectamente por la sustitución de especies provocada por la mortalidad asociada a *P. cinnamomi*.

Los resultados presentados en esta tesis doctoral han contribuido a mejorar nuestro conocimiento sobre las consecuencias del proceso de decaimiento y muerte del arbolado sobre el funcionamiento del suelo en bosques mediterráneos. El incremento de la

mortalidad del alcornoque es considerado un problema de gran envergadura para gestores, propietarios e investigadores debido a que provoca graves pérdidas tanto económicas como ecológicas y sociales. Esta tesis doctoral ha mostrado cómo, una vez el alcornoque es afectado y muere, el funcionamiento del bosque varía, produciéndose importantes cambios tanto en el reciclado de la materia orgánica como en la disponibilidad de nutrientes limitantes tan importantes como el fósforo y el nitrógeno.

GENERAL ABSTRACT

In the last decades, an increase in tree mortality rates has been detected worldwide. The main causes have been associated with global change drivers such as climate change and the outbreak of invasive insects and pathogens. The process of defoliation and death associated with these phenomena might have profound direct and indirect impacts on ecosystem functioning. However, our current knowledge about the consequences of an increase in tree mortality rates is still limited and strongly biased towards warming or insect-driven tree mortality. The aim of this doctoral thesis is to analyze the consequences on ecosystem functioning, and particularly on main biogeochemical cycles (carbon, nitrogen and phosphorus), of changes in the structure and composition of Mediterranean mixed forests in Southwestern Iberian Peninsula affected by the decline of its dominant species, *Quercus suber*, due to the invasive pathogen *Phytophthora cinnamomi*. The experimental works were undertaken in six study sites located in Los Alcornocales Natural Park (Cádiz). Three of the sites were situated in open woodlands dominated by *Q. suber* and *Olea europaea* var. *sylvestris* and the other three in closed forests of *Q. suber* and *Quercus canariensis*.

Chapter 2 focuses on the analysis of *Q. suber* tree capacity to adjust their physiology (water use efficiency and secondary growth) to pathogen-induced tree mortality across three scales: whole-tree, local and landscape scales. The results of this work showed how *Q. suber* trees affected by limiting edaphic factors and a high abundance of *P. cinnamomi* were able to increase their water use efficiency at local scale. However, this capacity was limited and might not be enough for the trees to overcome the physiological stress associated to the pathogen. Therefore, healthy and defoliated trees did not show differences in terms of water use efficiency or secondary branch growth.

The high vulnerability of *Q. suber* trees to the invasive pathogen might be the responsible of the high mortality that affects this species. Once the processes of defoliation and death begin, several changes on plant-soil relationships might trigger significant consequences on ecosystem functioning. In chapters 3 and 4, spatially-explicit neighbourhood models were used to assess the per capita effects of individual trees of different species and with different health status on key variables of the carbon, nitrogen and phosphorus cycles and on the microbial communities. These studies were performed at a large spatial (six study sites in two forest types) and temporal (intra- and inter-annual)

scales. This allowed to provide valuable insights in our knowledge about the consequences of tree mortality driven by pathogens on soil biology. **Chapter 3** describes the strong spatial concordance found among the distribution and health status of individual trees and soil respiration rates and nutrient availability. *Q. suber* decline led to a decrease in soil respiration rates and phosphorus availability, whereas its effects on nitrogen availability varied depending on forest types. In turn, **chapter 4** shows how *Q. suber* decline resulted in important changes of key variables of the carbon and nitrogen cycles, but the sign and magnitude of these effects varied depending on the local characteristics of the soil texture. *Q. suber* decline did not affect microbial functional diversity or biomass, but translated into lower soil microbial respiration. The results of both chapters showed strong differences in the footprint of *Q. suber* and coexistent tree species on carbon, nitrogen and phosphorus cycles. This might lead to important changes on the biogeochemical cycles in the long term if a replacement of *Q. suber* by other coexistent species not affected by decline occur during the process of secondary succession.

Chapter 5 analyzes how multiple global change drivers might affect simultaneously soil functioning in Mediterranean forests. Specifically, this work assesses the interactive effects of pathogen-driven *Q. suber* mortality, warming and drought on soil respiration and ammonium, nitrate and phosphorus availability. *P. cinnamomi*-induced mortality and future warming and drought may interact to simultaneously alter biogeochemical cycles in *Q. suber* forest soils. Moreover, this work showed that nutrient availability might be affected indirectly by *P. cinnamomi*-induced mortality due to species replacement.

The findings of this doctoral thesis have increased our knowledge about the consequences of the process of tree decline and mortality on the functioning of Mediterranean forest soils. The increase of *Q. suber* mortality is considered an important concern for managers, landowners and researchers not only due to the economic impact, but also the ecological and social losses that it inflicts. This doctoral thesis demonstrates how *Q. suber* decline affects forest functioning, with important changes in both the recycling of organic matters and the availability of limiting nutrients as important as phosphorus and nitrogen.

TABLA DE CONTENIDOS

CAPÍTULO 1

INTRODUCCIÓN GENERAL

INTRODUCCIÓN GENERAL

El decaimiento de bosques en todo el mundo: causas, patrones y vulnerabilidad

El proceso de la muerte de los árboles ha sido objeto de estudio durante décadas, fundamentalmente cuando se observan desviaciones en las tasas de mortalidad basales que se consideran normales en un bosque. Ya a finales del siglo XIX, varios trabajos describían fenómenos de decaimiento forestal y muerte por el ataque de insectos y enfermedades en distintos bosques del mundo (Farrer, 1883; Peringuey, 1884). A partir de los años 70 del siglo XX se extiende el concepto de decaimiento (*forest decline* en inglés o *Waldsterben* en alemán) para describir los fenómenos de mortalidad de árboles que se estaban detectando en Centro Europa y regiones de Norte América y Australia (Hinrichsen, 1987; Ciesla y Donaubauer, 1994). Estos trabajos trataban de explicar episodios de pérdida progresiva y prematura del vigor de árboles o masas de árboles sin síntomas evidentes de daño físico o ataque de enfermedades o plagas primarias (Ciesla y Donaubauer, 1994). Entre las causas más importante de estos fenómenos de decaimiento se situaban aquellas relacionadas con la contaminación del aire, como la lluvia ácida, el óxido nitroso, el ozono troposférico y la deposición de metales pesados (Mueller-Dombois, 1987; Ciesla y Donaubauer, 1994). Más recientemente, en las primeras décadas del siglo XXI, múltiples estudios han puesto de manifiesto el incremento en las tasas de mortalidad en bosques de todo el planeta (p. ej. van Mantgem *et al.*, 2009; Allen *et al.*, 2010; Wang *et al.*, 2012; IPCC, 2014; Hartmann *et al.*, 2018). Las causas asociadas a este fenómeno son complejas y están siendo objeto de estudio por la comunidad científica internacional (Martínez-Vilalta *et al.*, 2012; McDowell *et al.*, 2013). Sin embargo, frecuentemente se asocian a dos factores relacionados con el cambio global: (1) el cambio climático, bien directamente afectando a la fisiología del árbol, o bien interaccionando con el ciclo de vida de insectos y patógenos; y (2) las alteraciones bióticas asociadas a insectos y patógenos exóticos (Loo, 2009; Allen *et al.*, 2010; Gandhi y Herms, 2010; Carnicer *et al.*, 2011; Martínez-Vilalta *et al.*, 2012; Anderegg *et al.*, 2013; Lovett *et al.*, 2016).

El cambio climático ha sido identificado por múltiples estudios como un factor clave detrás del reciente incremento de la mortalidad arbórea detectado en bosques de diferentes

biomas (van Mantgem *et al.*, 2009; Adams *et al.*, 2010; Allen *et al.*, 2010; Carnicer *et al.*, 2011). Así, existen casos de mortalidad asociado al cambio climático en bosques templados del hemisferio norte (EE.UU, Canadá, Europa) y sur (Australia, Argentina), en regiones áridas y semiáridas (EE.UU, Europa), incluso en áreas tropicales de Asia y América (Allen *et al.*, 2010). La sequía y las altas temperaturas provocan la defoliación y muerte de los árboles por dos mecanismos interrelacionados: fallo hidráulico (*hydraulic failure*), que ocurre cuando la pérdida de agua por transpiración excede al aporte de agua por las raíces, provocando la cavitación en el xilema y la interrupción del transporte de agua; y por agotamiento de las reservas de carbono (*carbon starvation*), debido al cierre estomático para evitar la pérdida de agua y problemas de cavitación (McDowell *et al.*, 2008; McDowell, 2011). A esta mortalidad provocada por efectos directos del cambio climático sobre la fisiología del árbol, se le unen los efectos indirectos derivados del efecto positivo que el cambio climático (y particularmente el incremento de la temperatura) suele tener en plagas de insectos y patógenos (Allen *et al.*, 2010). Las altas temperaturas afectan directamente al ciclo de vida de insectos y microorganismos patógenos debido a que pueden afectar a su ciclo de vida, acelerando su desarrollo y reproducción y, por tanto, favoreciendo su capacidad de infección. Al mismo tiempo, las altas temperaturas y la sequía asociada funcionan como fuentes de estrés abiótico, reduciendo el vigor de los árboles y aumentando la susceptibilidad de éstos al ataque de insectos y patógenos (Ayres y Lombardero, 2000; Logan *et al.*, 2003; Desprez-Loustau *et al.*, 2006; Rouault *et al.*, 2006).

Junto al cambio climático, las invasiones de insectos y patógenos se sitúan como otra de las principales causas del aumento de la mortalidad arbórea (p. ej. Loo, 2009; Gandhi y Herms, 2010; Lovett *et al.*, 2010; Lovett *et al.*, 2016). El continuo flujo de insectos y patógenos invasores y la expansión de aquellos que ya están establecidos suponen una amenaza para los ecosistemas (Liebhold *et al.*, 2012; Lovett *et al.*, 2016). El incremento de las introducciones de especies exóticas puede, potencialmente, llevar asociado un incremento importante en el número de plagas. A pesar de que el número de especies exóticas que pueden causar plagas es menor al de especies nativas que podrían causarlas, los impactos ecológicos y económicos de las plagas asociadas a especies exóticas en los bosques son mucho más severos (Pimentel, 1986; Liebhold *et al.*, 1995). La severidad de estos impactos puede explicarse por la falta de historia co-evolutiva entre el hospedador y el insecto o patógeno exótico. Este hecho deja al hospedador sin defensas específicas efectivas lo que facilita el establecimiento, la dispersión e impacto del insecto o patógeno

invasor (Gandhi y Herms, 2010). Un claro ejemplo lo constituyen los oomicetos del género *Phytophthora*, que son reconocidos como patógenos primarios de miles de especies de árboles, matorrales y cultivos, causando grandes impactos ecológicos y económicos en todo el planeta (p. ej. Brasier, 1996; Erwin y Ribeiro, 1996; Jung *et al.*, 2000; Weste, 2003; Shearer *et al.*, 2009). Sin embargo, en la mayoría de los casos, los problemas de decaimiento asociado a especies del género *Phytophthora* solo ocurren cuando estas especies ocupan nuevos territorios y se vuelven invasoras, mientras que sus efectos en aquellos lugares donde este patógeno es nativo pasan desapercibidos (Jung *et al.*, 2018).

La mortalidad arbórea podría ser menor de lo esperado si los árboles disponen de mecanismos que les permitan atenuar los efectos negativos del cambio global, ya sea la sequía, el incremento de las temperaturas o el ataque de insectos y patógenos (Lloret *et al.*, 2012; Allen *et al.*, 2015). Por ejemplo, los árboles adaptados a ecosistemas áridos, pueden mostrar cierta capacidad fisiológica para tolerar sequías y altas temperaturas mediante un incremento en su eficiencia del uso del agua (Tenhunen *et al.*, 1990; Peñuelas *et al.*, 2000; Serrano y Peñuelas, 2005; Maseyk *et al.*, 2011). Sin embargo, bajo condiciones extremas, esta capacidad puede verse superada provocando la defoliación y muerte del árbol (Martínez-Vilalta *et al.*, 2002; McDowell *et al.*, 2008; McDowell, 2011). Los bosques tienen que enfrentarse cada vez más a la intensificación de factores estresantes que afectan a su salud, como el cambio climático, la contaminación del aire o las especies invasoras (Trumbore *et al.*, 2015). Un reciente estudio ha mostrado un incremento de la vulnerabilidad de las especies forestales a fenómenos de mortalidad asociados a cambios rápidos en el ambiente, como la sequía, independientemente del bioma en el que se encuentren, detectando que un 70% de las 226 especies forestales estudiadas tenían un limitado potencial fisiológico para responder a dichos cambios ambientales (Choat *et al.*, 2018). La intensificación de los factores de estrés que están provocando el aumento de la vulnerabilidad de especies forestales probablemente provoque que en las próximas décadas muchos bosques en diferentes ecosistemas sufran alteraciones y reorganizaciones asociados a estos fenómenos rápidos de cambio.

La mortalidad de árboles como determinante del funcionamiento del ecosistema

El proceso de defoliación y mortalidad asociado al fenómeno de decaimiento forestal puede tener grandes impactos directos en el funcionamiento del ecosistema y, concretamente, en las interacciones planta-suelo, pudiendo causar una reducción de la productividad primaria y cambios en la circulación de nutrientes a corto plazo (Lovett *et al.*, 2006; Hicke *et al.*, 2012; Wang *et al.*, 2012). A largo plazo, el incremento en las tasas de mortalidad podría indirectamente afectar a la función de todo el ecosistema si se produce un reemplazamiento de las especies debido a su diferente vulnerabilidad a los factores de mortalidad (Ellison *et al.*, 2005; Lovett *et al.*, 2010). A pesar del potencial que tiene el decaimiento forestal para producir efectos tanto directos como indirectos en el funcionamiento del ecosistema, nuestro conocimiento actual de las consecuencias del decaimiento es aún limitada y fuertemente sesgada a determinados eventos de mortalidad, como la asociada al incremento de las temperaturas o las plagas de insectos (ver revisiones de Hicke *et al.*, 2012; Wang *et al.*, 2012; y Anderegg *et al.*, 2013).

Los cambios que ocurren en el bosque a consecuencia del incremento de la tasa de mortalidad arbórea podrían traducirse en alteraciones de la comunidad microbiana y la actividad biológica del suelo. Por ejemplo, se han detectado descensos en la diversidad funcional microbiana en suelos bajo árboles en decaimiento, muy probablemente provocado por cambios en la cantidad y calidad de hojarasca o de los exudados radicales que podrían alterar la estructura de grupos funcionales (Cai *et al.*, 2010; Lloret *et al.*, 2015; Rodríguez *et al.*, 2016). También se ha detectado un incremento en la biomasa microbiana y la respiración heterótrofa asociado a la mortalidad de árboles (Edburg *et al.*, 2012; Zhang *et al.*, 2015). Sin embargo, otros trabajos no han encontrado efectos significativos de la mortalidad sobre las comunidades microbianas y sus funciones (p. ej. Holden y Treseder, 2013; Delaporte *et al.*, 2017), lo que podría indicar que estas relaciones son fuertemente contexto-dependiente. Hasta la fecha, muy pocos son los trabajos que se han centrado en estudiar los efectos de la mortalidad sobre la comunidad microbiana, por lo que serían necesarios más estudios para poder predecir el efecto de la mortalidad sobre la abundancia, diversidad y funcionamiento de las comunidades microbianas bajo diferentes escenarios de cambio global.

El ciclo de carbono también podría ser alterado drásticamente por un incremento en las tasas de mortalidad arbórea (Kurz *et al.*, 2008; Hicke *et al.*, 2012; Anderegg *et al.*,

2013). Los efectos de la mortalidad de árboles sobre el ciclo del carbono son complejos ya que pueden afectar a diferentes componentes (flujos y reservas) del mismo. Algunos estudios han detectado descensos en las reservas de carbono tras fenómenos de mortalidad (Xiong *et al.*, 2011; Hicke *et al.*, 2012), mientras que un reciente meta-análisis ha señalado que estos descensos afectan a las reservas de carbono con tiempos de residencia relativamente altos, mientras que tienden a aumentar aquellas formas de carbono más lábiles (Zhang *et al.*, 2015). Respecto a los flujos de carbono, la defoliación y la muerte de los árboles podrían provocar un descenso de la actividad de las raíces que desencadene una disminución en la respiración autótrofa del suelo (respiración de raíces y la rizosfera asociada) (Nuckolls *et al.*, 2009; Nave *et al.*, 2011). Sin embargo, la mortalidad también podría provocar incrementos en la respiración heterótrofa (asociada a los microorganismos) debido a aumentos en la cantidad de hojarasca o cambios en el microclima bajo árboles defoliados y muertos (Edburg *et al.*, 2012; Zhang *et al.*, 2015). Por tanto, el efecto de la mortalidad arbórea en la respiración del suelo dependerá del balance entre su efecto sobre la actividad de las raíces y la respiración microbiana (e.g. Morehouse *et al.*, 2008; Hicke *et al.*, 2012).

No solo el carbono puede verse alterado por la defoliación y mortalidad de árboles, sino que ésta también podría tener efectos severos en nutrientes tan importantes para el funcionamiento del bosque como el nitrógeno y el fósforo. Sin embargo, pocos trabajos hasta la fecha han estudiado el efecto del incremento de la mortalidad sobre los nutrientes del suelo, lo que dificulta poder predecir el signo y la magnitud de dichos efectos y los factores que los determinan. Durante la defoliación y muerte de un árbol se podrían esperar cambios en la cantidad y calidad de la hojarasca, así como un descenso en la absorción de nutrientes que podrían provocar incrementos en el nitrógeno tanto orgánico como inorgánico bajo estos árboles (Hobara *et al.*, 2001; Kizlinski *et al.*, 2002; Stadler *et al.*, 2005; Morehouse *et al.*, 2008; Edburg *et al.*, 2012). Además, las condiciones microclimáticas bajo los árboles en decaimiento podrían también modificar la actividad de los microorganismos, traduciéndose en una alteración de las tasas de mineralización y afectando a la cantidad de nitrógeno disponible bajo estos árboles (Mladenoff, 1987; Scharenbroch y Bockheim, 2007). Estos incrementos observados para el nitrógeno podrían en principio esperarse también para el fósforo. Sin embargo, la mortalidad de árboles implica una reducción drástica de los mecanismos que las plantas utilizan para aumentar la disponibilidad de fósforo orgánico y mineral en el suelo (enzimas fosfatasas, ácidos orgánicos; ver Jurinak *et al.*, 1986; Hinsinger, 2001; Schneider *et al.*, 2001; George

et al., 2011), lo que podría inclinar la balanza hacia una menor disponibilidad de este nutriente bajo árboles defoliados y muertos.

El fenómeno de incremento de mortalidad suele estar caracterizado por mostrar un cierto nivel de especificidad, afectando a algunas especies y no a otras (Lovett *et al.*, 2006; Ibáñez *et al.*, 2017). Como resultado, se pueden observar cambios en la abundancia relativa de las especies, de tal forma que la especie afectada reducirá el área que ocupa en el bosque a favor de especies co-existentes no afectadas por el incremento de la mortalidad. Si las especies co-dominantes no afectadas son funcionalmente diferentes a la especie afectada, a largo plazo el impacto indirecto del decaimiento sobre el funcionamiento del ecosistema podría ser mayor que los efectos directos provocados a corto plazo por la defoliación y mortalidad de los árboles (Hancock *et al.*, 2008; Lovett *et al.*, 2010; Barba *et al.*, 2013). Por ejemplo, Lovett *et al.* (2010) observaron importantes cambios a largo plazo en los ciclos de C y N en bosques afectados por la enfermedad de la corteza del haya (*beech bark disease*) debido a la sustitución de la especie afectada, *Fagus grandifolia*, por la especie coexistente *Acer saccharum*, mientras que los efectos directos de la muerte de la especie afectada fueron mínimos debido principalmente a la baja velocidad del proceso de mortalidad. De forma similar, Cobb *et al.* (2013) encontraron que en bosques de California afectados por el patógeno *Phytophthora ramorum*, el mayor impacto sobre el ciclo del nitrógeno estaba asociado a la sustitución de la especie afectada, *Notholithocarpus densiflorus,* por la especie coexistente *Umellularia californica*, debido al mayor contenido en nitrógeno de la hojarasca de la especie sustituta respecto a la especie afectada por el patógeno.

En resumen, el incremento en las tasas basales de mortalidad de árboles y los picos de mortalidad asociadas a eventos extremos (ej. sequías) podrían alterar severamente las comunidades microbianas y los ciclos de carbono, nitrógeno y fósforo del bosque. Además, estos efectos podrían revertir en cambios aún mayores sobre la comunidad de plantas en un proceso de retroalimentación que puede ser tanto positivo como negativo (Gómez-Aparicio *et al.*, 2017). Sin embargo, la información existente sobre cómo los ciclos de materia y energía varían en ecosistemas afectados por decaimiento es escasa y nos puede inducir a realizar predicciones imprecisas sobe sus consecuencias para el funcionamiento del ecosistema. Por todo ello, se hace fundamental conocer en profundidad cómo los procesos ecosistémicos varían en función de la abundancia, tamaño y distribución espacial de las distintas especies y su estado de salud en ecosistemas afectados por decaimiento.

El fenómeno de la seca en los ecosistemas mediterráneos

Los bosques formados por especies del género *Quercus* se encuentran entre los más afectados por fenómenos de decaimiento forestal. El decaimiento de especies de *Quercus* (*oak decline*) afecta a numerosos bosques en diferentes sistemas de Europa y Norte América (Brasier, 1996; Thomas *et al.*, 2002; McConnell y Balci, 2014; Bendixsen *et al.*, 2015; Haavik *et al.*, 2015). Concretamente en la península ibérica, el decaimiento de especies de *Quercus* (conocido popularmente como "la seca"), se ha detectado fundamentalmente en bosques de *Quercus ilex* (encina) y *Quercus suber* (alcornoque) en Andalucía occidental, Extremadura y sur de Portugal. Este fenómeno es de suma importancia en estas regiones porque afecta a dehesas y montados, sistemas agro-forestales gestionados para la explotación ganadera, forestal, cinegética y agrícola con superficies de pastos y monte mediterráneo con un gran valor ecológico, económico y social (Aronson *et al.*, 2009). El decaimiento de especies de *Quercus* se ha definido tradicionalmente como un proceso de etiología compleja que conlleva una combinación de varios factores abióticos y bióticos actuando de forma combinada, para provocar la defoliación y muerte del árbol (Tuset y Sánchez, 2004; Carrasco *et al.*, 2009; Camilo-Alves *et al.*, 2013). Sin embargo, en 1991 se aisló por primera vez al agresivo patógeno exótico *Phytophthora cinnamomi* de raíces finas de individuos enfermos de *Q. ilex* y *Q. suber* en el centro y sur de la península ibérica (Brasier, 1992; Brasier, 1996). Desde entonces, se ha situado a este patógeno como el principal agente causante de este fenómeno de decaimiento, muy probablemente en interacción con factores climáticos, edáficos, otros agentes bióticos y las prácticas de gestión forestal (Camilo-Alves *et al.*, 2013). Por ejemplo, la precipitación anual (especialmente la precipitación en verano y otoño) y los periodos de sequía afectan en gran medida a la incidencia de la enfermedad, a los síntomas que presentan los árboles afectados y a la distribución de la misma (Tuset y Sánchez, 2004; Bendixsen *et al.*, 2015). Asimismo, también se ha visto que características del suelo como altos niveles de arcilla y poca profundidad pueden predisponer al árbol a la defoliación y muerte (Costa *et al.*, 2010; Corcobado *et al.*, 2013).

P. cinnamomi es considerada como una de las especies invasoras más peligrosas del planeta (Lowe *et al.*, 2000). Su origen se sitúa muy probablemente en la zona suroccidental del Océano Pacífico (Papúa Nueva Guinea-Celebes) desde donde se ha expandido durante los siglos XIX y XX (Jung *et al.*, 2016), encontrándose hoy en día ampliamente distribuida en regiones templadas y tropicales de todo el mundo (Burgess *et*

al., 2017). Este patógeno, aunque en determinadas condiciones puede presentar capacidades saprófitas, suele alimentarse de tejido vegetal vivo, atacando a las raíces absorbentes de los árboles y, en menor medida, a la corteza de raíces principales y el tronco (Zentemeyer, 1980; Weste y Marks, 1987). Cuando el tiempo es cálido y húmedo, se forman los esporangios a partir de la germinación de clamidosporas (estructuras de resistencia) o del propio micelio del oomiceto. Los esporangios producen y liberan las zoosporas (esporas infectivas biflageladas) que, transportadas por el agua del suelo, pueden infectar a otras raíces (Weste y Marks, 1987). *P. cinnamomi* es un patógeno generalista con más de 1000 huéspedes conocidos, pero con un amplio rango de susceptibilidad entre ellos (Erwin y Ribeiro, 1996), siendo *Q. ilex* y *Q. suber* las dos especies del género *Quercus* más susceptibles de la península ibérica (Maurel *et al.*, 2001; Moreira y Martins, 2005). Una vez los árboles son infectados muestran unos síntomas secundarios similares a los ocasionados por la sequía: clorosis, marchitez de hojas, secado de ramas y ausencia de raíces absorbentes (Sánchez *et al.*, 2003). Tras el ataque del patógeno se puede producir un decaimiento lento, que puede conllevar varios años, donde se observa una caída progresiva de las hojas y la presencia de ramas parcial o totalmente defoliadas. O bien, puede producirse una muerte súbita, caracterizada por un rápido secado de las hojas (meses), que suelen permanecer durante un tiempo adheridas al árbol (Gallego *et al.*, 1999).

Concretamente, en alcornocales del sur de la península ibérica se está observando una pérdida de la principal especie formadora del dosel, el *Q. suber*, atribuida principalmente al patógeno exótico *P. cinnamomi* (Sánchez *et al.*, 2002). Dicho proceso de decaimiento no parece afectar a otras especies coexistentes, como el quejigo (*Quercus canariensis*) o el acebuche (*Olea europaea* var. *sylvestris*). El incremento de la mortalidad de *Q. suber* puede provocar efectos a corto plazo en las comunidades microbianas del bosque, así como en los ciclos biogeoquímicos. Además, son de esperar cambios importantes a medio-largo plazo en la composición del dosel de estos bosques hacia una pérdida de dominancia de *Q. suber* que podrían conllevar importantes modificaciones de los procesos edáficos. Sin embargo, hasta ahora no existe suficiente información para poder conocer y predecir con precisión las consecuencias de la mortalidad de *Q. suber* asociada a patógenos invasores en el funcionamiento del ecosistema.

Más allá de *P. cinnamomi*: otras amenazas de los bosques mediterráneos e interacción entre factores

Además del efecto de los patógenos invasores, los bosques mediterráneos también tendrán que hacer frente al fenómeno de cambio climático (Lindner *et al.*, 2010; Doblas-Miranda *et al.*, 2015). La cuenca mediterránea está sufriendo un descenso de las precipitaciones y un fuerte incremento de la temperatura y de la severidad de las sequías en los últimos veinte años (Peñuelas *et al.*, 2002; Vicente-Serrano *et al.*, 2014). Además, los modelos climáticos predicen un incremento de la aridez para las próximas décadas en esta región (Wetherald y Manabe, 2002; Planton *et al.*, 2012). Estas condiciones climáticas probablemente puden conllevar una reducción en el crecimiento de las plantas y de la productividad primaria, así como en la dinámica de la comunidad de plantas y su composición (Ogaya *et al.*, 2003; Matías *et al.*, 2011). Este incremento de la aridez podría ocasionar también cambios en el funcionamiento del suelo, pudiendo incrementar la mineralización de la materia orgánica y la liberación de nutrientes (Emmett *et al.*, 2004; Delgado-Baquerizo *et al.*, 2013). Sin embargo, el signo y magnitud del impacto de la sequía y el calentamiento sobre estas variables no está suficientemente demostrado (Emmett *et al.*, 2004; Sardans *et al.*, 2008).

La interacción del efecto de los patógenos con un incremento de la temperatura y la aridez podría provocar efectos sobre el funcionamiento del ecosistema de forma hasta ahora impredecible. Cuando múltiples factores de estrés afectan a un ecosistema simultáneamente, el resultado final podría ser mayor o menor a la simple adición de efectos de cada factor si ellos actuaran de forma independiente (Paine *et al.*, 1998). La realización de experimentos que analicen los efectos interactivos de diferentes factores de estrés sobre el funcionamiento del ecosistema nos permitiría ayudar a comprender las consecuencias que estos múltiples factores podrían tener en el ecosistema y que actualmente no podemos obtener a partir de una aproximación de análisis de un único factor (Dieleman *et al.*, 2012; Zhou *et al.*, 2016; Yue *et al.*, 2017). A pesar de que muchos estudios se han centrado en analizar el impacto del cambio climático en los ecosistemas, hasta el momento no se ha estudiado experimentalmente el efecto del cambio climático en el funcionamiento del bosque en sistemas afectados por seca, lo que nos impide predecir el impacto de estos fenómenos a futuro.

Aproximación metodológica para el estudio de las consecuencias del decaimiento sobre procesos del suelo: modelos de vecindad y modelos lineales

Para analizar el efecto de las especies arbóreas o del estado de salud de los árboles sobre procesos del suelo se podría acudir a la aproximación más frecuentemente utilizada en el estudio de relaciones planta-suelo y que se basa en la comparación de los efectos medios de distintas especies en rodales monoespecíficos o bajo árboles aislados de cada especie o estado de salud (microhábitats). Estas aproximaciones permiten analizar y aislar el efecto de una especie (o estado de salud) en concreto. Sin embargo, en los sistemas naturales, las plantas crecen entremezcladas unas con otras, y es la combinación del efecto de individuos de distintas especies el que afecta a las características del suelo en un determinado punto. Por ello, los resultados obtenidos mediante estas aproximaciones deberían ser tomados con cautela en sistemas naturales donde las copas se solapan. Para poder analizar el efecto de individuos correspondientes a distintas especies de árboles o con distintos estados de salud que crecen de forma entremezclada, en esta tesis doctoral se han aplicado los **modelos de vecindad**. Esta aproximación permite relacionar la dinámica de poblaciones con los procesos ecosistémicos de forma espacialmente explícita (Canham *et al.*, 2006; Gómez-Aparicio y Canham, 2008; Boyden *et al.*, 2012). En esta tesis doctoral estos modelos han permitido relacionar procesos y características del suelo tales como la respiración o la disponibilidad de nutrientes con la distribución espacial de los árboles en la vecindad.

Los modelos de vecindad estiman el valor de una propiedad ecosistémica en un punto *p* a partir de las características propias del punto *p* y una función que cuantifica las características de la vecindad o entorno inmediato de dicho punto. Esta función puede tomar múltiples formas funcionales y puede incorporar diferentes propiedades de los árboles en la vecindad del punto *p:* abundancia de vecinos, tamaño, distancia al punto, especie, estado de salud, etc. (Gómez-Aparicio *et al.*, 2013). En esta tesis doctoral el efecto de los árboles sobre las variables edáficas se midió usando un índice de vecindad -NI, del inglés *Neighbourhood Index*. Este índice cuantifica el efecto neto de $j = 1, \ldots, n$ árboles vecinos de $i = 1, \ldots, s$ especies sobre las variables del suelo. Es directamente proporcional al tamaño del árbol vecino (*dbh* o diámetro a la altura del pecho) e inversamente proporcional a la distancia (*d*) que separa el árbol al punto de muestreo (Boyden *et al.*, 2012; Ibáñez *et al.*, 2015):

$$NI = \sum_{i=0}^{s} \cdot \sum_{j=0}^{n} \lambda_i \frac{dbh_{ij}^{\alpha}}{d_{ij}^{\beta}}$$

En esta fórmula, *NI* es el índice de vecindad, dbh_{ij} el diámetro a la altura del pecho del árbol *j* de la especie *i*, d_{ij} es la distancia entre el árbol vecino *j* de la especie *i* y el punto de muestreo, y α y β son parámetros que estiman la intensidad del efecto del tamaño del árbol y la distancia, respectivamente. Finalmente, estos modelos permiten caracterizar la "huella" de árboles de distintas especies o estados de salud sobre las propiedades del ecosistema, tanto en términos de magnitud como de alcance espacial (i.e. cómo el efecto desaparece con la distancia al árbol) gracias al coeficiente per cápita λ, que varía en función de la especie o estado de salud árbol vecino objeto de estudio.

Para aplicar los modelos de vecindad en esta tesis doctoral se utiliza la aproximación desarrollada por Canham y Uriarte (2006), aplicando métodos de máxima verosimilitud y comparación de modelos. Esta aproximación aporta mucha flexibilidad ya que permite ajustar y comparar modelos que usan diferentes características de los árboles y formas funcionales para seleccionar aquellos que mejor se ajusten a la realidad y, por tanto, que tengan un mayor soporte empírico (Gómez-Aparicio *et al.*, 2013). Una vez se definen los distintos modelos, se realiza una estimación de los parámetros de los modelos mediante métodos de optimización global y se usa el criterio de información de Akaike (AIC) para elegir el modelo con un mayor soporte empírico (Burnham y Anderson, 2002). Para realizar estos análisis se ha desarrollado un programa específico en lenguaje R usando la librería *likelihood* y contando con el asesoramiento del Prof. Dr. Charles Canham (Cary Institute of Ecosystem Studies, Nueva York, EEUU).

Para aquellos trabajos de esta tesis doctoral que no tenían un diseño espacialmente explícito se ha aplicado otro tipo de análisis estadístico, los **modelos lineales generalizados**. Concretamente esta aproximación estadística se ha utilizado para analizar las relaciones entre determinadas variables fisiológicas y el estado de salud de los árboles, así como para analizar el posible efecto interactivo entre diferentes factores sobre los procesos del suelo. Para el ajuste de estos modelos se ha seleccionado la función de distribución más adecuada a los datos utilizados en cada caso. Así mismo, se han utilizado **modelos lineales generalizados mixtos** cuando ha sido necesaria la incorporación de variables aleatorias a los modelos (Crawley, 2007; Zuur *et al.*, 2009). Estas aproximaciones permiten poder modelar variables que no cumplen los presupuestos matemáticos de los modelos estadísticos más tradicionales, como el modelo lineal

general. Además, los modelos lineales generalizados y los modelos lineales generalizados mixtos permiten obtener una más adecuada representación de la realidad ya que ofrece un marco adecuado para que tanto los criterios de ajuste, como los de parsimonia e integración teórica exigibles al modelo, puedan irse conformando (Crawley, 2007; Faraway, 2016).

Sitio de estudios y especies

Esta tesis doctoral se ha realizado en bosques mixtos mediterráneos localizados en el parque natural Los Alcornocales, en las provincias de Cádiz y Málaga. El parque natural Los Alcornocales está localizado dentro de la zona biogeográfica Bético-Rifeña, que ha sido considerada como uno de los puntos calientes (*hot spots*) de biodiversidad de la cuenca mediterránea (Medail y Quezel, 1997). El clima en el parque natural está muy condicionado por su posición geográfica. Las temperaturas medias están muy suavizadas, situadas en torno a los 15.7ºC, alcanzándose los máximos en los meses de verano (entre 22 y 26ºC), mientras que los valores medios invernales se encuentran entre los 7 y los 10ºC. Las precipitaciones son abundantes y a veces torrenciales, asociadas a los vientos de poniente, aumentando hacia el interior y con la altura. El parque natural esté situado entre las isoyetas 600 y 2100 mm, con una precipitación media anual de 1014 mm (AEMET, 2011).

La vegetación del parque natural está dominada por formaciones boscosas esclerófilas de *Q. suber*, localizadas fundamentalmente sobre los sustratos ácidos de las areniscas del Aljibe en altitudes comprendidas entre los 100-700 m. En las zonas bajas con predominio de suelos arcillosos, *Q. suber* aparece formando bosques abiertos (*open woodlands*) entremezclado con *Olea europaea* var. *sylvestris* (acebuche). Estas formaciones suelen tener un sotobosque muy variado, pudiendo ser en ocasiones muy denso. El sotobosque está formado por *Phillyrea latifolia, Phillyrea angustifolia, Pistacia lentiscus, Cistus salvifolius*, *Myrtus communis*, *Crataegus monogyna, Genista linifolia*, *Ulex borgiae, Cytisus striatus, Erica scoparia* y *Quercus coccifera* (Ibarra, 1993). En los fondos de valle y en laderas más frescas y umbrías, *Q. suber* forma bosques cerrados (*closed forests*) entremezclado con el quejigo (*Quercus canariensis*). Estos bosques suelen estar acompañado de un sotobosque con un alto grado de estructuración vertical formado por especies arbustivas como *Arbutus unedo, Viburnum tinus, Erica arborea, Erica australis, Cistus salvifolius, Myrtus communis, Ruscus hypophyllum* y *Rubus*

ulmifolius. Suelen ser abundantes las especies de musgos, líquenes y epífitos, así como especies del estrato lianoide (*Smilax aspera, Hedera helix, Lonicera periclymenum*) y helechos como *Pteridium aquilinum* y *Asplenium onopteris* (Pérez-Ramos, 2006). En las últimas décadas, los bosques de *Q. suber* del parque natural sufren severos problemas de decaimiento forestal asociado al patógeno invasor *Phytophthora cinnamomi* (Brasier, 1992; Sánchez *et al.*, 2002; Gómez-Aparicio *et al.*, 2008). La principal especie afectada es *Q. suber*, mientras que el resto de especies coexistentes como el acebuche y el quejigo o el matorral del sotobosque, no parecen estar afectados.

Para los trabajos de campo de esta tesis doctoral se establecieron seis parcelas de estudio de 1 ha en zonas afectadas por decaimiento asociado a *P. cinnamomi*. Tres de las parcelas se situaron en bosques mixtos de *Q. suber* y *O. europaea* (bosque abierto, *open woodlands*): Picacho, Cinchao y Ahumada. Las otras tres parcelas se situaron en bosques mixtos de *Q. suber* y *Q. canariensis* (bosque cerrado, *closed forest*): Tala, Jimena y Comares (Figuras 1 y 2).

Figura 1 Situación de las parcelas de estudio en el parque natural Los Alcornocales (rodeado en verde en el mapa).

Figura 2 Fotografías de un bosque abierto (*open woodland*) a la izquierda y un bosque cerrado (*closed forest*) a la derecha en el parque natural Los Alcornocales.

En cada una de las parcelas de estudio se estableció una cuadrícula de 70 x 70 m^2 con puntos de muestreo situados regularmente cada 10 metros (n = 49 puntos por parcela, 294 puntos en total). En dichos puntos se realizaron todas las mediciones de los procesos y variables biogeoquímicas del suelo. Asimismo, se mapearon todos los árboles y matorrales en la vecindad de cada punto, definiéndose la vecindad como un radio de 15 metros en torno a cada punto (Figura 3). De cada individuo vecino se registró la especie y el tamaño y, en el caso de *Q. suber*, se diferenciaron árboles con distintos estados de salud (sanos, afectados por decaimiento y muertos). Para aquellos trabajos en los que no se aplicó una aproximación espacialmente explícita, se seleccionaron individuos dentro de las parcelas de estudio o en su proximidad, evitando el solapamiento de la copa con otros árboles y matorrales.

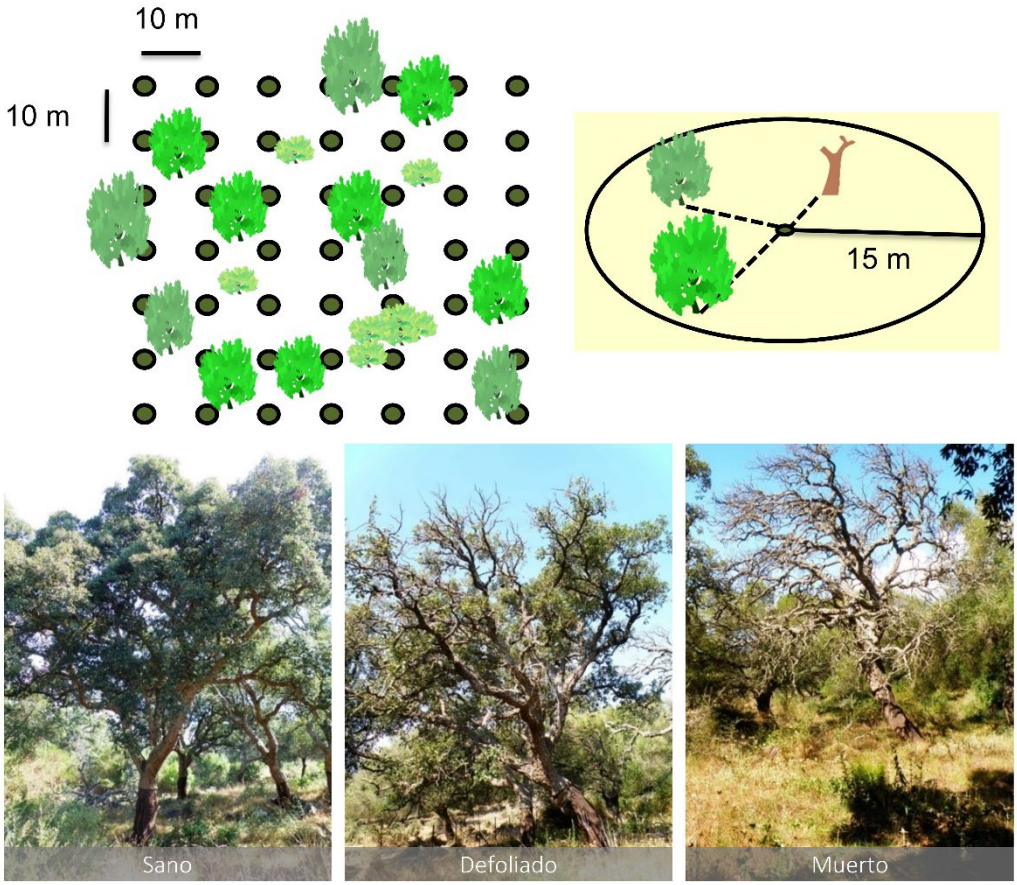

Figura 3 Esquema de los puntos de muestreo situados en cada una de las parcelas de estudio. Se indican los círculos empleados para definir la vecindad arbórea (en negro, 15 m de radio). Se muestran individuos de *Q. suber* con distinto estado de salud.

Objetivo, hipótesis y estructura de la tesis

Esta tesis doctoral tiene como **objetivo general** analizar las consecuencias para el funcionamiento del ecosistema, y particularmente para los principales ciclos biogeoquímicos (carbono, nitrógeno y fósforo), de cambios en la estructura y composición de bosques mixtos del sur de la península ibérica asociados al decaimiento de su especie dominante, el *Quercus suber*, debido al patógeno exótico *Phytophthora cinnamomi*. Esta tesis representa un novedoso esfuerzo por conectar comunidades complejas formadas por árboles y matorrales de diferentes especies, tamaños y estados de salud, con la variabilidad espacial y temporal de procesos y características del suelo, permitiendo la estimación del efecto per cápita de los árboles en el ecosistema en un contexto de decaimiento forestal. Además, esta tesis pretende analizar la interacción del efecto del patógeno invasor *P. cinnamomi* y otros factores de cambio global como la sequía y el incremento de las temperaturas en los procesos del suelo. En conjunto, los

resultados de esta tesis doctoral se pretenden aportar un conocimiento científico sólido que permita conocer en profundidad, interpretar y pronosticar la dinámica del bosque mediterráneo, contribuyendo a su conservación, gestión y restauración en el escenario actual de cambio global.

Esta tesis doctoral se ha organizado por capítulos en formato de artículos científicos. Tras la Introducción General (Capítulo 1), se presentan cuatro capítulos en inglés (Capítulos 2 a 5) cada uno con sus secciones Resumen, Introducción, Material y Métodos, Resultados y Discusión. A cada uno de estos capítulos le antecede un resumen en español. Los capítulos 2, 3 y 5 se encuentran publicados en *European Journal of Forest Research*, *Journal of Ecology* y *Biogeochemisrty*, respectivamente, mientras que el capítulo 4 se encuentra en revisión en *New Phytologist*. La tesis doctoral finaliza con el Capítulo 6 en el que se desarrolla una discusión general de los principales resultados obtenidos y el Capítulo 7 que incluye las conclusiones generales. Tras cada capítulo se incluyen las referencias de la literatura citada. A continuación, se detallan el contenido y los objetivos de los capítulos científicos 2 a 5.

En el **capítulo 2** se analiza si los árboles de *Q. suber* afectados por decaimiento asociado a *P. cinnamomi* son capaces de ajustar su fisiología, en términos de eficiencia del uso del agua y crecimiento secundario, a diferentes escalas espaciales: individual, local y regional. Específicamente, en este trabajo se estudió si árboles defoliados mostraban diferente eficiencia en el uso del agua y tasa de crecimiento secundario que árboles sanos a escala de individuo. Posteriormente, se analizó como a escala local, factores edáficos abióticos y bióticos a escala local (profundidad del suelo, potencial hidráulico, textura, materia orgánica, abundancia de *P. cinnamomi*) pueden explicar diferencias en la eficiencia del uso del agua y el crecimiento secundario de árboles sanos y defoliados. Finalmente, se analizaron diferencias en la eficiencia del uso del agua y crecimiento secundario a escala regional. Para responder a estas preguntas, se seleccionaron individuos de *Q. suber* sanos y defoliados en los 6 sitios de estudio distribuidos por el parque natural Los Alcornocales (Cádiz), 3 localizados en bosque abierto y 3 en bosque cerrado. Las hipótesis en este capítulo fueron:

1) A escala individual, los árboles defoliados tendrán una mayor eficiencia del uso del agua y menor crecimiento secundario que árboles sanos.
2) A escala local, una mayor eficiencia del uso del agua y un menor crecimiento secundario estarán relacionados con propiedades físico-químicas limitantes del suelo y una mayor abundancia de *P. cinnamomi*.

3) A escala regional, la eficiencia del uso del agua y el crecimiento secundario de los árboles variarán entre tipos de bosque con diferentes características edafoclimáticas.

En los **capítulos 3 y 4** se utiliza la aproximación de vecindad espacialmente explícita para ajustar modelos basados en estimadores de máxima verosimilitud donde determinadas variables clave de ciclos biogeoquímicos y de las comunidades microbianas son analizadas en función de la abundancia y distribución de los árboles del dosel en las seis parcelas de estudio situadas en los dos tipos de bosques estudiados (bosque abierto y bosque cerrado). En el **capítulo 3** se muestra cómo el decaimiento de *Q. suber* modifica los ciclos de carbono, nitrógeno y fósforo en bosques mixtos mediterráneos afectados por *P. cinnamomi*. En este trabajo se utilizaron modelos de vecindad para predecir cómo la respiración del suelo, y la disponibilidad de nitrógeno y fósforo varían en función del tamaño, identidad, estado de salud, abundancia y distribución de árboles adultos en la vecindad. Se analizaron los efectos directos del decaimiento de *Q. suber* al comparar el impacto de individuos de *Q. suber* con diferente estado de salud, así como los potenciales efectos indirectos a largo plazo, al comparar el impacto de especies coexistentes que no sufren decaimiento. Además, en este trabajo se analizaron posibles diferencias intra- e inter-anuales en el efecto de las especies del dosel y el estado de salud en los ciclos biogeoquímicos. Concretamente, el estudio se realizó en 2 estaciones contrastadas (primavera y verano), para poder estudiar las diferencias intra-anuales que pueden ocurrir debido a la variación en el contenido hídrico del suelo entre estas estaciones. Además, el estudio se repitió en 3 años sucesivos con contrastadas condiciones meteorológicas para analizar posibles variaciones inter-anuales en el efecto de las especies del dosel y el estado de salud en los ciclos biogeoquímicos. Se comprobaron las siguientes hipótesis:

1) El decaimiento *de Q. suber* asociado a *P. cinnamomi* causará reducciones en las tasas de respiración del suelo y en la disponibilidad de fósforo, pero incrementos en la disponibilidad de nitrógeno.
2) La huella de las especies arbóreas coexistentes, *O. europaea* y *Q. canariensis*, sobre la respiración del suelo y la disponibilidad de nutrientes será diferente a la huella de *Q. suber*.
3) Los efectos del decaimiento de *Q. suber* en los ciclos biogeoquímicos será de mayor magnitud en primavera que en verano. La magnitud de estos efectos variará entre años con marcadas diferencias en la precipitación anual.

En el **capítulo 4** se vuelven a aplicar modelos de vecindad espacialmente explícitos, esta vez para analizar el efecto del decaimiento de *Q. suber* en la diversidad, abundancia y funcionamiento de las comunidades microbianas y en componentes clave de los ciclos del carbono y nitrógeno. Concretamente en este trabajo se caracterizaron y compararon los efectos per cápita de los individuos de *Q. suber* con distinto estado de salud sobre la diversidad funcional, la biomasa y la respiración de la comunidad microbiana. Además, se estudió el efecto sobre diferentes componentes clave del ciclo del carbono como el carbono total del suelo, el carbono orgánico disuelto y componentes importantes de la materia orgánica disuelta (hexosas, pentosas, aminoácidos y fenoles) y del ciclo del nitrógeno, como el nitrógeno orgánico disuelto, el amonio y el nitrato. Finalmente, se evaluaron los potenciales efectos indirectos a largo plazo del decaimiento de *Q. suber* al comparar el efecto per cápita de individuos sanos de *Q. suber* con individuos de otras especies del dosel no afectados por decaimiento. Se estudiaron las siguientes hipótesis:

1) El decaimiento de *Q. suber* provocará un descenso de la diversidad funcional microbiana y un aumento de la biomasa microbiana y la respiración heterótrofa del suelo.
2) El decaimiento de *Q. suber* disminuirá los compuestos de carbono con altos tiempos de residencia relativamente altos, mientras que aumentará aquellas formas de carbono más lábiles.
3) Tanto el nitrógeno orgánico como el orgánico aumentarán como consecuencia del decaimiento de *Q. suber*.
4) Los impactos del decaimiento de *Q. suber* variarán entre tipos de bosques con diferentes características texturales del suelo.
5) Las huellas de las especies arbóreas coexistentes diferirán respecto a la huella de *Q. suber* en las variables estudiadas.

En el **capítulo 5** se pretende comprender cómo múltiples factores de cambio global afectan simultáneamente al funcionamiento del suelo en ecosistemas mediterráneos. Concretamente, se estudian los efectos del decaimiento de *Q. suber* asociado a *P. cinnamomi*, el calentamiento, la sequía y sus interacciones en variables clave de ciclos biogeoquímicos. Para ello se realizó un experimento de incubaciones en laboratorio a partir de suelos recogidos bajo individuos de *Q. suber* con diferente estado de salud y de especies de matorral coexistentes no afectadas por decaimiento. Estos suelos fueron

incubados bajo condiciones controladas de temperatura y humedad simulando varios escenarios climáticos predichos para la región mediterránea. En este trabajo se analizan los efectos interactivos del decaimiento, la sequía y el calentamiento sobre la respiración del suelo y la disponibilidad de amonio, nitrato y fosfato. Además, se analizó la resistencia de estas variables del suelo a sufrir cambios bajo determinados escenarios de temperatura y humedad predichos para 2050 y 2100. Finalmente, se muestra el efecto indirecto que podría tener el decaimiento de *Q. suber* si los espacios dejados por los árboles muertos son ocupados por matorral sucesional coexistente no afectado por decaimiento. Las hipótesis que se estudiaron en este capítulo fueron:

1) El efecto del incremento de la temperatura y descenso de la humedad del suelo predichos como consecuencia del cambio climático podría interaccionar con el efecto del decaimiento de *Q. suber* sobre variables clave de los ciclos de carbono, nitrógeno y fósforo.
2) La resistencia de la respiración del suelo y la disponibilidad de nutrientes a sufrir cambios bajo determinados escenarios de temperatura y humedad predichos para 2050 y 2100 será diferente en suelos bajo árboles afectados por decaimiento que bajo árboles sanos.
3) El impacto del incremento de la temperatura y el descenso de la humedad será diferente en suelos bajo matorral que en suelos bajo *Q. suber.*

Referencias

Adams HD, Macalady AK, Breshears DD, Allen CD, Stephenson NL, Saleska SR, Huxman TE. 2010. Climate-Induced Tree Mortality: Earth System Consequences. *Eos, Transactions American Geophysical Union* 91(17): 153-154.

AEMET 2011. Atlas climático ibérico. Temperatura del aire y precipitación 1971–2000. Agencia Estatal de Meteorología: Ministerio de Medio Ambiente y Medio Rural y Marino.

Allen CD, Breshears DD, McDowell NG. 2015. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. *Ecosphere* 6(8): 129.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim JH, Allard G, Running SW, Semerci A,

Cobb N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259(4): 660-684.

Anderegg WRL, Kane JM, Anderegg LDL. 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. *Nature Climate Change* 3(1): 30-36.

Aronson J, Pausas JG, Pereira JS. 2009. Cork oak woodlands on the edge: Ecology, adaptive management, and restoration: Island Press.

Ayres MP, Lombardero MaJ. 2000. Assessing the consequences of global change for forest disturbance from herbivores and pathogens. *Science of the Total Environment* 262(3): 263-286.

Barba J, Curiel Yuste J, Martínez-Vilalta J, Lloret F. 2013. Drought-induced tree species replacement is reflected in the spatial variability of soil respiration in a mixed Mediterranean forest. *Forest Ecology and Management* 306: 79-87.

Bendixsen DP, Hallgren SW, Frazier AE. 2015. Stress factors associated with forest decline in xeric oak forests of south-central United States. *Forest Ecology and Management* 347: 40-48.

Boyden S, Montgomery R, Reich PB, Palik B. 2012. Seeing the forest for the heterogeneous trees: stand-scale resource distributions emerge from tree-scale structure. *Ecological Applications* 22(5): 1578-1588.

Brasier CM. 1992. Oak tree mortality in Iberia. *Nature* 360(6404): 539.

Brasier CM. 1996. *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. *Annals of Forest Science* 53(2-3): 347-358.

Burgess TI, Scott JK, Mcdougall KL, Stukely MJC, Crane C, Dunstan WA, Brigg F, Andjic V, White D, Rudman T, Arentz F, Ota N, Hardy GESJ. 2017. Current and projected global distribution of Phytophthora cinnamomi, one of the world's worst plant pathogens. *Global Change Biology* 23(4): 1661-1674.

Burnham KP, Anderson DR. 2002. Model Selection and Multi-Model Inference : A Practical Information-Theoretic Approach. Secaucus, NJ, USA: Springer.

Cai YF, Barber P, Dell B, O'Brien P, Williams N, Bowen B, Hardy G. 2010. Soil bacterial functional diversity is associated with the decline of Eucalyptus gomphocephala. *Forest Ecology and Management* 260(6): 1047-1057.

Camilo-Alves CSP, da Clara MIE, Ribeiro NA. 2013. Decline of Mediterranean oak trees and its association with *Phytophthora cinnamomi*: a review. *European Journal of Forest Research* 132(3): 411-432.

Canham CD, Papaik MJ, Uriarte M, McWilliams WH, Jenkins JC, Twery MJ. 2006. Neighborhood analyses of canopy tree competition along environmental gradients in New England forests. *Ecological Applications* 16(2): 540-554.

Canham CD, Uriarte M. 2006. Analysis of neighborhood dynamics of forest ecosystems using likelihood methods and modeling. *Ecological Applications* 16(1): 62-73.

Carnicer J, Coll M, Ninyerola M, Pons X, Sánchez G, Peñuelas J. 2011. Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* 108(4): 1474-1478.

Carrasco Á, Fernández Á, Trapero A, López G, Sánchez I, Ruiz JM, Jiménez JJ, Domínguez L, Romero MdlÁ, Carbonero MD, Sánchez ME, Lucas PC, Gil P, Fernández P, Navarro RM, Sánchez R, Raposo R, Rodríguez S. 2009. *Procesos de decaimiento forestal (la Seca), situación del conocimiento*. Córdoba: Consejería de Medio Ambiente, Junta de Andalucía.

Ciesla WM, Donaubauer E. 1994. *Decline and dieback of trees and forests: a global overview*: Food & Agriculture Organization of the UN (FAO).

Cobb RC, Eviner VT, Rizzo DM. 2013. Mortality and community changes drive sudden oak death impacts on litterfall and soil nitrogen cycling. *New Phytologist* 200(2): 422-431.

Corcobado T, Solla A, Madeira M, Moreno G. 2013. Combined effects of soil properties and *Phytophthora cinnamomi* infections on *Quercus ilex* decline. *Plant and Soil* 373(1-2): 403-413.

Costa A, Pereira H, Madeira M. 2010. Analysis of spatial patterns of oak decline in cork oak woodlands in Mediterranean conditions. *Annals of Forest Science* 67(2): 204 (201-210).

Crawley MJ. 2007. *The R book*. UK: Wiley.

Choat B, Brodribb TJ, Brodersen CR, Duursma RA, Lopez R, Medlyn BE. 2018. Triggers of tree mortality under drought. *Nature* 558(7711): 531-539.

Delaporte A, Zanella A, Vincent G, Bugeat M, Damesin C, Bazot S. 2017. Structural and functional differences in the belowground compartment of healthy and declining beech trees. *Applied Soil Ecology* 117-118: 106-116.

Delgado-Baquerizo M, Maestre FT, Gallardo A, Bowker MA, Wallenstein MD, Quero JL, Ochoa V, Gozalo B, Garcia-Gomez M, Soliveres S, Garcia-Palacios P, Berdugo M, Valencia E, Escolar C, Arredondol T, Barraza-Zepeda C, Bran D, Carreiral JA, Chaiebll M, Conceicao AA, Derak M, Eldridge DL, Escudero A, Espinosa CI, Gaitan J, Gatica MG, Gomez-Gonzalez S, Guzman E, Gutierrez JR, Florentino A, Hepper E, Hernandez RM, Huber-Sannwald E, Jankju M, Liu JS, Mau RL, Miriti M, Monerris J, Naseri K, Noumi Z, Polo V, Prina A, Pucheta E, Ramirez E, Ramirez-Collantes DA, Romao R, Tighe M, Torres D, Torres-Diaz C, Ungar ED, et al. 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. *Nature* 502(7473): 672-676.

Desprez-Loustau ML, Marcais B, Nageleisen LM, Piou D, Vannini A. 2006. Interactive effects of drought and pathogens in forest trees. *Annals of Forest Science* 63(6): 597-612.

Dieleman WIJ, Vicca S, Dijkstra FA, Hagedorn F, Hovenden MJ, Larsen KS, Morgan JA, Volder A, Beier C, Dukes JS, King J, Leuzinger S, Linder S, Luo Y, Oren R, De Angelis P, Tingey D, Hoosbeek MR, Janssens IA. 2012. Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO2 and temperature. *Global Change Biology* 18(9): 2681-2693.

Doblas-Miranda E, Martínez-Vilalta J, Lloret F, Álvarez A, Ávila A, Bonet F, Brotons L, Castro J, Curiel Yuste J, Díaz M. 2015. Reassessing global change research priorities in mediterranean terrestrial ecosystems: how far have we come and where do we go from here? *Global Ecology and Biogeography* 24(1): 25-43.

Edburg SL, Hicke JA, Brooks PD, Pendall EG, Ewers BE, Norton U, Gochis D, Gutmann ED, Meddens AJH. 2012. Cascading impacts of bark beetle-caused tree mortality on coupled biogeophysical and biogeochemical processes. *Frontiers in Ecology and the Environment* 10(8): 416-424.

Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppel BD, Knoepp JD, Lovett GM, Mohan J, Orwig DA, Rodenhouse NL, Sobczak WV, Stinson KA, Stone JK, Swan CM, Thompson J, Von Holle B, Webster JR. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3(9): 479-486.

Emmett BA, Beier C, Estiarte M, Tietema A, Kristensen HL, Williams D, Peñuelas J, Schmidt I, Sowerby A. 2004. The response of soil processes to climate change:

Results from manipulation studies of shrublands across an environmental gradient. *Ecosystems* 7(6): 625-637.

Erwin DC, Ribeiro OK. 1996. *Phytophthora Diseases Worldwide*. St Paul, Minnesota: APS Press.

Faraway JJ. 2016. Extending the linear model with R: generalized linear, mixed effects and nonparametric regression models: CRC press.

Farrer J. 1883. The Age of Trees. *Longman's magazine, 1882-1905* 2(11): 524-532.

Gallego FJ, de Algaba AP, Fernandez-Escobar R. 1999. Etiology of oak decline in Spain. *European Journal of Forest Pathology* 29(1): 17-27.

Gandhi KJK, Herms DA. 2010. Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. *Biological Invasions* 12(2): 389-405.

George TS, Fransson A-M, Hammond JP, White PJ 2011. Phosphorus Nutrition: Rhizosphere Processes, Plant Response and Adaptations. In: Bünemann E, Oberson A, Frossard E eds. *Phosphorus in Action*: Springer Berlin Heidelberg, 245-271.

Gómez-Aparicio L, Ávila J, Cayuela L. 2013. Métodos de máxima verosimilitud en ecología y su aplicación en modelos de vecindad. *Ecosistemas* 22(3): 12-20.

Gómez-Aparicio L, Canham CD. 2008. Neighborhood models of the effects of invasive tree species on ecosystem processes. *Ecological Monographs* 78(1): 69-86.

Gómez-Aparicio L, Domínguez-Begines J, Kardol P, Ávila JM, Ibáñez B, García LV. 2017. Plant-soil feedbacks in declining forests: implications for species coexistence. *Ecology* 98(7): 1908-1921.

Gómez-Aparicio L, Zamora R, Castro J, Hódar JA. 2008. Facilitation of tree saplings by nurse plants: Microhabitat amelioration or protection against herbivores? *Journal of Vegetation Science* 19(2): 161-172.

Haavik LJ, Billings SA, Guldin JM, Stephen FM. 2015. Emergent insects, pathogens and drought shape changing patterns in oak decline in North America and Europe. *Forest Ecology and Management* 354: 190-205.

Hancock JE, Arthur MA, Weathers KC, Lovett GM. 2008. Carbon cycling along a gradient of beech bark disease impact in the Catskill Mountains, New York. *Canadian Journal of Forest Research* 38(5): 1267-1274.

Hartmann H, Schuldt B, Sanders TGM, Macinnis-Ng C, Boehmer HJ, Allen CD, Bolte A, Crowther TW, Hansen MC, Medlyn BE, Ruehr NK, Anderegg WRL. 2018.

Monitoring global tree mortality patterns and trends. Report from the VW symposium 'Crossing scales and disciplines to identify global trends of tree mortality as indicators of forest health'. *New Phytologist* 217(3): 984-987.

Hicke JA, Allen CD, Desai AR, Dietze MC, Hall RJ, Hogg EH, Kashian DM, Moore D, Raffa KF, Sturrock RN, Vogelmann J. 2012. Effects of biotic disturbances on forest carbon cycling in the United States and Canada. *Global Change Biology* 18(1): 7-34.

Hinrichsen D. 1987. The forest decline enigma. *Bioscience* 37(8): 542-546.

Hinsinger P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil* 237(2): 173-195.

Hobara S, Tokuchi N, Ohte N, Koba K, Katsuyama M, Kim SJ, Nakanishi A. 2001. Mechanism of nitrate loss from a forested catchment following a small-scale, natural disturbance. *Canadian Journal of Forest Research* 31(8): 1326-1335.

Holden SR, Treseder KK. 2013. A meta-analysis of soil microbial biomass responses to forest disturbances. *Frontiers in Microbiology* 4.

Ibáñez B, Gómez-Aparicio L, Ávila JM, Pérez-Ramos IM, Marañón T. 2017. Effects of Quercus suber Decline on Woody Plant Regeneration: Potential Implications for Successional Dynamics in Mediterranean Forests. *Ecosystems* 20(3): 630-644.

Ibáñez B, Gómez-Aparicio L, Stoll P, Ávila JM, Pérez-Ramos IM, Marañón T. 2015. A neighborhood analysis of the consequences of *Quercus suber* decline for regeneration dynamics in Mediterranean forests. *PloS one* 10(2): e0117827.

Ibarra P. 1993. Naturaleza y hombre en el sur del Campo de Gibraltar: un análisis paisajístico integrado. Sevilla: Agencia de Medio Ambiente.

IPCC. 2014. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jung T, Blaschke H, Oßwald W. 2000. Involvement of soilborne Phytophthora species in Central European oak decline and the effect of site factors on the disease. *Plant Pathology* 49(6): 706-718.

Jung T, Orlikowski L, Henricot B, Abad-Campos P, Aday AG, Aguín Casal O, Bakonyi J, Cacciola SO, Cech T, Chavarriaga D, Corcobado T, Cravador A, Decourcelle T, Denton G, Diamandis S, Doğmuş-Lehtijärvi HT, Franceschini A, Ginetti B, Green S, Glavendekić M, Hantula J, Hartmann G, Herrero M, Ivic D, Horta Jung

M, Lilja A, Keca N, Kramarets V, Lyubenova A, Machado H, Magnano di San Lio G, Mansilla Vázquez PJ, Marçais B, Matsiakh I, Milenkovic I, Moricca S, Nagy ZÁ, Nechwatal J, Olsson C, Oszako T, Pane A, Paplomatas EJ, Pintos Varela C, Prospero S, Rial Martínez C, Rigling D, Robin C, Rytkönen A, Sánchez ME, Sanz Ros AV, et al. 2016. Widespread *Phytophthora* infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of Phytophthora diseases. *Forest Pathology* 46(2): 134-163.

Jung T, Pérez-Sierra A, Durán A, Jung MH, Balci Y, Scanu B. 2018. Canker and decline diseases caused by soil-and airborne Phytophthora species in forests and woodlands. *Persoonia* 40: 182-220.

Jurinak JJ, Dudley LM, Allen MF, Knight WG. 1986. The role of calcium-oxalate in the availability of phosphorus in soils of semiarid regions - a thermodynamic study. *Soil Science* 142(5): 255-261.

Kizlinski ML, Orwig DA, Cobb RC, Foster DR. 2002. Direct and indirect ecosystem consequences of an invasive pest on forests dominated by eastern hemlock. *Journal of Biogeography* 29(10-11): 1489-1503.

Kurz WA, Dymond CC, Stinson G, Rampley GJ, Neilson ET, Carroll AL, Ebata T, Safranyik L. 2008. Mountain pine beetle and forest carbon feedback to climate change. *Nature* 452(7190): 987-990.

Liebhold AM, Brockerhoff EG, Garrett LJ, Parke JL, Britton KO. 2012. Live plant imports: the major pathway for forest insect and pathogen invasions of the US. *Frontiers in Ecology and the Environment* 10(3): 135-143.

Liebhold AM, Macdonald WL, Bergdahl D, Maestro VC. 1995. INVASION BY EXOTIC FOREST PESTS - A THREAT TO FOREST ECOSYSTEMS. *Forest Science* 41(2): 1-49.

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M. 2010. Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecology and Management* 259(4): 698-709.

Logan JA, Regniere J, Powell JA. 2003. Assessing the impacts of global warming on forest pest dynamics. *Frontiers in Ecology and the Environment* 1(3): 130-137.

Loo JA. 2009. Ecological impacts of non-indigenous invasive fungi as forest pathogens. *Biological Invasions* 11(1): 81-96.

Lovett GM, Arthur MA, Weathers KC, Griffin JM. 2010. Long-term changes in forest carbon and nitrogen cycling caused by an introduced pest/pathogen complex. *Ecosystems* 13(8): 1188-1200.

Lovett GM, Canham CD, Arthur MA, Weathers KC, Fitzhugh RD. 2006. Forest ecosystem responses to exotic pests and pathogens in eastern North America. *Bioscience* 56(5): 395-405.

Lovett GM, Weiss M, Liebhold AM, Holmes TP, Leung B, Lambert KF, Orwig DA, Campbell FT, Rosenthal J, McCullough DG. 2016. Nonnative forest insects and pathogens in the United States: Impacts and policy options. *Ecological Applications* 26(5): 1437-1455.

Lowe S, Browne M, Boudjelas S, De Poorter M. 2000. *100 of the world's worst invasive alien species: a selection from the global invasive species database.*: Invasive Species Specialist Group Species Survival Commission, World Conservation Union (IUCN), Auckland, New Zealand.

Lloret F, Escudero A, Iriondo JM, Martínez-Vilalta J, Valladares F. 2012. Extreme climatic events and vegetation: the role of stabilizing processes. *Global Change Biology* 18(3): 797-805.

Lloret F, Mattana S, Curiel Yuste J. 2015. Climate-induced die-off affects plant–soil–microbe ecological relationship and functioning. *FEMS Microbiology Ecology* 91(2): 1-12.

Martínez-Vilalta J, Lloret F, Breshears DD. 2012. Drought-induced forest decline: causes, scope and implications. *Biology Letters* 8(5): 689-691.

Martínez-Vilalta J, Prat E, Oliveras I, Piñol J. 2002. Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. *Oecologia* 133(1): 19-29.

Maseyk K, Hemming D, Angert A, Leavitt SW, Yakir D. 2011. Increase in water-use efficiency and underlying processes in pine forests across a precipitation gradient in the dry Mediterranean region over the past 30 years. *Oecologia* 167(2): 573-585.

Matías L, Zamora R, Castro J. 2011. Repercussions of Simulated Climate Change on the Diversity of Woody-Recruit Bank in a Mediterranean-type Ecosystem. *Ecosystems* 14(4): 672-682.

Maurel M, Robin C, Capron G, Desprez-Loustau ML. 2001. Effects of root damage associated with *Phytophthora cinnamomi* on water relations, biomass

accumulation, mineral nutrition and vulnerability to water deficit of five oak and chestnut species. *Forest Pathology* 31(6): 353-369.

McConnell ME, Balci Y. 2014. Phytophthora cinnamomi as a Contributor to White Oak Decline in Mid-Atlantic United States Forests. *Plant Disease* 98(3): 319-327.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* 178(4): 719-739.

McDowell NG. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiology* 155(3): 1051-1059.

McDowell NG, Ryan MG, Zeppel MJB, Tissue DT. 2013. Improving our knowledge of drought-induced forest mortality through experiments, observations, and modeling. *New Phytologist* 200(2): 289-293.

Medail F, Quezel P. 1997. Hot-spots analysis for conservation of plant biodiversity in the Mediterranean Basin. *Annals of the Missouri Botanical Garden*: 112-127.

Mladenoff DJ. 1987. Dynamics of nitrogen mineralization and nitrification in hemlock and hardwood treefall gaps. *Ecology* 68(5): 1171-1180.

Morehouse K, Johns T, Kaye J, Kaye A. 2008. Carbon and nitrogen cycling immediately following bark beetle outbreaks in southwestern ponderosa pine forests. *Forest Ecology and Management* 255(7): 2698-2708.

Moreira AC, Martins JMS. 2005. Influence of site factors on the impact of Phytophthora cinnamomi in cork oak stands in Portugal. *Forest Pathology* 35(3): 145-162.

Mueller-Dombois D. 1987. Natural dieback in forests. *Bioscience* 37(8): 575-583.

Nave LE, Gough CM, Maurer KD, Bohrer G, Hardiman BS, Le Moine J, Munoz AB, Nadelhoffer KJ, Sparks JP, Strahm BD, Vogel CS, Curtis PS. 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. *Journal of Geophysical Research-Biogeosciences* 116: G04016.

Nuckolls AE, Wurzburger N, Ford CR, Hendrick RL, Vose JM, Kloeppel BD. 2009. Hemlock declines rapidly with hemlock woolly adelgid infestation: impacts on the carbon cycle of Southern Appalachian forests. *Ecosystems* 12(2): 179-190.

Ogaya R, Peñuelas J, Martínez-Vilalta J, Mangirón M. 2003. Effect of drought on diameter increment of Quercus ilex, Phillyrea latifolia, and Arbutus unedo in a holm oak forest of NE Spain. *Forest Ecology and Management* 180(1-3): 175-184.

Paine RT, Tegner MJ, Johnson EA. 1998. Compounded perturbations yield ecological surprises. *Ecosystems* 1(6): 535-545.

Peñuelas J, Filella I, Comas P. 2002. Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. *Global Change Biology* 8(6): 531-544.

Peñuelas J, Filella I, Lloret F, Piñol J, Siscart D. 2000. Effects of a Severe Drought on Water and Nitrogen Use by *Quercus ilex* and *Phyllyrea latifolia*. *Biologia Plantarum* 43(1): 47-53.

Pérez-Ramos IM. 2006. Factores que condicionan la regeneración natural de especies leñosas en un bosque mediterráneo del sur de la Península Ibérica. Universidad de Sevilla Sevilla.

Peringuey L. 1884. Insects injurious to forest trees in South Africa. *Transactions of the South African Philosophical Society* 4(1): 15-25.

Pimentel D 1986. Biological invasions of plants and animals in agriculture and forestry. *Ecology of biological invasions of North America and Hawaii*: Springer, 149-162.

Planton S, Lionello P, Artale V, Aznar R, Carrillo A, Colin J, Congedi L, Dubois C, Elizalde A, Gualdi S, Hertig E, Jacobeit J, Jordà G, Li L, Mariotti A, Piani C, Ruti P, Sanchez-Gomez E, Sannino G, Sevault F, Somot S, Tsimplis M 2012. The Climate of the Mediterranean Region in Future Climate Projections. In: Lionello P ed. *The Climate of the Mediterranean Region*. Oxford: Elsevier, 449-502.

Rodríguez A, Curiel Yuste J, Rey A, Durán J, García-Camacho R, Gallardo A, Valladares F. 2016. Holm oak decline triggers changes in plant succession and microbial communities, with implications for ecosystem C and N cycling. *Plant and Soil*: 1-17.

Rouault G, Candau J-N, Lieutier F, Nageleisen L-M, Martin J-C, Warzée N. 2006. Effects of drought and heat on forest insect populations in relation to the 2003 drought in Western Europe. *Ann. For. Sci.* 63(6): 613-624.

Sánchez ME, Caetano P, Ferraz J, Trapero A. 2002. Phytophthora disease of *Quercus ilex* in south-western Spain. *Forest Pathology* 32(1): 5-18.

Sánchez ME, Sánchez JE, Navarro RM, Fernández P, Trapero A. 2003. Incidencia de la podredumbre radical causada por *Phytophtora cinnamomi* en masas de *Quercus* en Andalucía. *Boletín de sanidad vegetal. Plagas.* 29(1): 87-108.

Sardans J, Peñuelas J, Estiarte M. 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. *Applied Soil Ecology* 39(2): 223-235.

Scharenbroch BC, Bockheim JG. 2007. Impacts of forest gaps on soil properties and processes in old growth northern hardwood-hemlock forests. *Plant and Soil* 294(1-2): 219-233.

Schneider K, Turrión MB, Grierson PF, Gallardo JF. 2001. Phosphatase activity, microbial phosphorus, and fine root growth in forest soils in the Sierra de Gata, western central Spain. *Biology and Fertility of Soils* 34(3): 151-155.

Serrano L, Peñuelas J. 2005. Contribution of physiological and morphological adjustments to drought resistance in two Mediterranean tree species. *Biologia Plantarum* 49(4): 551-559.

Shearer BL, Crane CE, Fairman RG, Dunne CP. 2009. Ecosystem dynamics altered by pathogen-mediated changes following invasion of Banksia woodland and *Eucalyptus marginata* forest biomes of south-western Australia by *Phytophthora cinnamomi*. *Australasian Plant Pathology* 38(4): 417-436.

Stadler B, Müller T, Orwig D, Cobb R. 2005. Hemlock woolly adelgid in New England forests: Canopy impacts transforming ecosystem processes and landscapes. *Ecosystems* 8(3): 233-247.

Tenhunen J, Serra AS, Harley P, Dougherty R, Reynolds JF. 1990. Factors influencing carbon fixation and water use by Mediterranean sclerophyll shrubs during summer drought. *Oecologia* 82(3): 381-393.

Thomas F, Blank R, Hartmann G. 2002. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. *Forest Pathology* 32(4-5): 277-307.

Trumbore S, Brando P, Hartmann H. 2015. Forest health and global change. *Science* 349(6250): 814-818.

Tuset JJ, Sánchez G. 2004. *La Seca: El decaimiento de encinas, alcornoques y otros Quercus en España*. Madrid: Ministerio de Medio Ambiente. Organismo Autónomo de Parques Nacionales.

van Mantgem PJ, Stephenson NL, Byrne JC, Daniels LD, Franklin JF, Fule PZ, Harmon ME, Larson AJ, Smith JM, Taylor AH, Veblen TT. 2009. Widespread increase of tree mortality rates in the western United States. *Science* 323(5913): 521-524.

Vicente-Serrano SM, Lopez-Moreno JI, Beguería S, Lorenzo-Lacruz J, Sanchez-Lorenzo A, García-Ruiz JM, Azorin-Molina C, Morán-Tejeda E, Revuelto J, Trigo R, Coelho F, Espejo F. 2014. Evidence of increasing drought severity caused by temperature rise in southern Europe. *Environmental Research Letters* 9(4): 044001.

Wang WF, Peng CH, Kneeshaw DD, Larocque GR, Luo ZB. 2012. Drought-induced tree mortality: ecological consequences, causes, and modeling. *Environmental Reviews* 20(2): 109-121.

Weste G. 2003. The dieback cycle in Victorian forests: a 30-year study of changes caused by Phytophthora cinnamomi in Victorian open forests, woodlands and heathlands. *Australasian Plant Pathology* 32(2): 247-256.

Weste G, Marks GC. 1987. The biology of *Phytophthora cinnamomi* in australasian forests. *Annual Review of Phytopathology* 25: 207-229.

Wetherald RT, Manabe S. 2002. Simulation of hydrologic changes associated with global warming. *Journal of Geophysical Research-Atmospheres* 107(D19): 4379.

Xiong Y, D'Atri JJ, Fu S, Xia H, Seastedt TR. 2011. Rapid soil organic matter loss from forest dieback in a subalpine coniferous ecosystem. *Soil Biology and Biochemistry* 43(12): 2450-2456.

Yue K, Fornara DA, Yang W, Peng Y, Peng C, Liu Z, Wu F. 2017. Influence of multiple global change drivers on terrestrial carbon storage: additive effects are common. *Ecology Letters* 20(5): 663-672.

Zentemeyer GA. 1980. *Phytophthora cinnamomi and the diseases it causes*. St. Paul, Minn.: American Phytopathological Society.

Zhang B, Zhou X, Zhou L, Ju R. 2015. A global synthesis of below-ground carbon responses to biotic disturbance: a meta-analysis. *Global Ecology and Biogeography* 24(2): 126-138.

Zhou L, Zhou X, Shao J, Nie Y, He Y, Jiang L, Wu Z, Hosseini Bai S. 2016. Interactive effects of global change factors on soil respiration and its components: a meta-analysis. *Global Change Biology* 22(9): 3157-3169.

Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM. 2009. *Mixed Effects Models and Extensions in Ecology with R*: Springer.

ACROSS-SCALE PATTERNING OF PLANT-SOIL-PATHOGEN INTERACTIONS IN *QUERCUS SUBER* DECLINE

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Across-scale patterning of plant-soil-pathogen interactions in *Quercus suber* decline

Resumen

Se ha detectado que bosques localizados en distintas regiones del planeta están siendo afectados por un severo decaimiento y elevadas tasas de mortalidad. A pesar de ello, nuestro conocimiento sobre el proceso de decaimiento a diferentes escalas es aún limitado. En este trabajo se ha analizado cómo los árboles de *Quercus suber* ajustan su fisiología, en términos de eficiencia del uso del agua y crecimiento secundario, ante un decaimiento asociado al patógeno *Phytophthora cinnamomi* a diferentes escalas: individual, local y regional. Este trabajo se realizó en bosques mixtos mediterráneos donde la especie dominante *Q. suber* está afectada por un intenso decaimiento y mortalidad relacionada con el patógeno exótico *P. cinnamomi*. No se detectaron diferencias significativas en la eficiencia en el uso del agua ni en el crecimiento secundario entre árboles defoliados y sanos. Sin embargo, se encontró que condiciones limitantes, tales como suelos con poca profundidad y una alta abundancia de *P. cinnamomi*, se relacionan con árboles con mayores eficiencias en el uso del agua a escala local y regional. En general, los resultados de este trabajo mostraron que los árboles de *Q. suber* sometidos a factores edáficos limitantes y patógenos de raíces incrementan su eficiencia en el uso del agua hasta cierto punto, aunque esta respuesta podría no ser suficiente para que los árboles superen el estrés fisiológico asociado al decaimiento inducido por el patógeno. En el presente trabajo se discute la compleja respuesta fisiológica de árboles de *Q. suber* adultos a la mortalidad inducida por *P. cinnamomi*, lo que mejora nuestro conocimiento sobre las posibles consecuencias del decaimiento crónico de *Quercus* en el futuro.

Abstract

Forests worldwide have been recently affected by severe decline and mortality, while our understanding about forest decline across spatial scale is still limited. In this work we study how *Quercus suber* trees adjust their physiology, in terms of water use efficiency and secondary growth, to pathogen-induced oak decline at the whole-tree, local and landscape scales. This work was carried out in Mediterranean mixed forests where their dominant key species *Q. suber* is affected by a severe decline and mortality induced by the exotic soil-borne pathogen *Phytophthora cinnamomi*. Significant differences were not observed between defoliated and healthy trees, either in terms of water use efficiency or growth at the whole-tree scale. We found that limiting conditions, such as low soil depth and high pathogen abundance, induced trees to higher water use efficiency at local and landscape scales. Overall our findings point out that *Q. suber* trees subjected to soil drought and root pathogens increase water use efficiency to some extent, while this response might not be enough for the trees to overcome the physiological stress associated to the pathogen-induced dieback. We discuss the complex way by which adult *Q. suber* trees physiologically respond to *P. cinnamomi*-induced mortality, improving our understanding of the likely consequences of chronic oak decline in the future.

Keywords

Carbon isotope, cork oak, cross-scale relationship, defoliation, ecophysiology, exotic pathogen, forest decline, forest disease, root pathogen, secondary growth, soil heterogeneity, tree dieback.

Introduction

In the recent decades, forests worldwide have been affected by severe decline and mortality (e.g. van Mantgem et al., 2009; Brouwers et al., 2013; Allen et al., 2015). In these affected systems, environmental stress operating at individual scale (e.g. physiological tree stress) can directly cause tree mortality, but also can interact with other disturbance processes at local or intermediate scales (e.g. insect pests). The result of such interactions may trigger complex responses subjected to significant uncertainties (Levin, 1992; Chave, 2013) that can be key drivers of the ecosystem dynamics (Allen, 2007; Peters et al., 2007). Nonetheless, our understanding of forest decline across spatial scales is constrained by limited knowledge of species-specific physiological thresholds, individual- and site-specific variation in these mortality thresholds and pathogen (or insect)-related feedbacks (Allen, 2007).

In a context of worldwide tree mortality, oak forests have been severely impaired in a number of different systems in Europe and North America (e.g. Thomas et al., 2002; Haavik et al., 2015). Oak decline is considered of paramount importance in Mediterranean systems because this phenomenon involves evergreen oak species (e.g. *Quercus ilex, Quercus suber, Quercus agrifolia*) that dominate the typical agroforestry systems of the west Mediterranean Basin (i.e. *dehesas* and *montados*) and oak savannas of western North America. Evergreen oak trees are a major structural element in these forests and maintain ecosystem functions and services such as enhancing diversity, soil protection, provisioning of resources and cultural services (Marañón et al., 1999; Olea & San Miguel-Ayanz, 2006; Marañón et al., 2012). In the Mediterranean Basin the decline of these keystone species is frequently caused by soil-borne *Phytophthora* species (Brasier, 1992; Pérez-Sierra et al., 2013; Jung et al., 2016). Although several species of *Phytophthora* have been isolated in soils from declining Mediterranean oak forests, *Phytophthora cinnamomi* is by far the most aggressive one and has been consistently recognized as a main driver of oak mortality in central and southern Spain, Portugal and southern regions of France and Italy (Brasier, 1992; Sánchez et al., 2002; Corcobado et al., 2014). This soil-borne pathogen that attacks tree root systems is considered one of the 100 most dangerous invasive species on earth (Weste & Marks, 1987; Lowe et al., 2000). Oak decline involves the interaction of *P. cinnamomi* with abiotic drivers that can modulate the effects of the pathogen on trees at different spatial scales (Brasier, 1996; Balci et al., 2010; Camilo-Alves et al., 2013). For example, climatic factors such as drought and heat

weaves have been identified as important drivers of oak decline that can accelerate the oak mortality process at regional scale (e.g. Camilo-Alves et al., 2013; Corcobado et al., 2014; Bendixsen et al., 2015). Moreover, other abiotic factors such as soil characteristics can influence trees at local scale, weakening and predisposing trees to defoliation and death (Costa et al., 2010; Camilo-Alves et al., 2013; Corcobado et al., 2013). The high socio-economic and environmental value of evergreen oaks in Mediterranean systems highlights the need to understand the drivers of oak decline at different scales.

Tree mortality can be lower than expected when individuals have the capability to attenuate the detrimental effects of stress (Lloret et al., 2012). It has been shown that seedlings and saplings of evergreen *Quercus* species may respond physiologically to *P. cinnamomi* infection in ways similar to drought stress, at least in terms of water relationships. For instance, previous studies have found that infected *Q. suber* and *Q. ilex* seedlings showed lower stomatal conductance and, in turn, higher water use efficiency than non-infected ones (Luque et al., 1999; Maurel et al., 2001; Robin et al., 2001; Sghaier-Hammami et al., 2013). However, these studies were performed under controlled watering regimes in greenhouse experiments. To our knowledge, there is a lack of information about physiological responses to *P. cinnamomi* infection in adult *Q. suber* trees under field conditions. If adult trees cannot show any responsive mechanisms or the effective physiological response is exceeded, a high mortality rate might be expected, which in turn could trigger cascading effects at ecosystem level (Ellison et al., 2005; Edburg et al., 2012; Anderegg et al., 2013). For example, a decrease in *Q. suber* recruitment has been detected in Mediterranean forests affected by *P. cinnamomi*, that might alter the successional trajectories of Mediterranean oak forests (Ibáñez et al., 2015). *Q. suber* decline is also affecting ecosystem functioning, reducing soil respiration and decoupling limiting nutrients (Ávila et al., 2016), which might limit even further demographic and ecosystem processes in Mediterranean forests. Moreover, how trees respond physiologically to pathogen attack can trigger regional-scale massive decline (Allen, 2007). These complex relationships require the study of ecological processes at different spatial scales. In this context, characterizing the response of key species to current pathogen-driven tree mortality processes may provide valuable information for predicting future community-level responses in a context of global change, as well as to improve conservation and management guidelines.

Here we aim to analyze how individuals of the dominant evergreen oak species *Quercus suber* adjust their physiology, in terms of water use efficiency and secondary

growth, at different scales in Mediterranean oak forests affected by pathogen-induced oak decline. First, we explored whether defoliated trees have different water use efficiency and secondary growth than healthy trees at the whole tree-scale (i.e. differences attributable to each individual tree). Second, we assessed which abiotic (i.e. soil depth, organic matter, water content and texture) and biotic factors (i.e. pathogen abundance) explain tree water use efficiency and secondary growth for healthy and defoliated *Q. suber* trees at the local scale (i.e. comparing trees with different microsite conditions). Finally, we analyzed differences in water use efficiency and secondary growth at landscape scale (i.e. comparing trees in different forest types). We hypothesized that (1) defoliated trees would show higher water use efficiency and lower secondary growth than healthy trees; (2) higher water use efficiency and lower secondary growth would be related to limiting soil abiotic conditions and pathogen abundance and (3) at landscape scale, water use efficiency and tree growth would vary among forest types differing in edaphoclimatic characteristics.

Methods

Study area and tree species

This study was carried out in mixed oak forests in Los Alcornocales Natural Park (Southern Spain), one of the largest *Q. suber* forests within the Mediterranean Basin (Urbieta *et al.*, 2008). The climate is sub-humid Mediterranean with warm, dry summers and mild, humid winters. Annual mean temperature ranges from 15.4 to 17.3 ºC and annual mean rainfall varies from 720 to 1100 mm (period 1951-1999, Ninyerola *et al.*, 2005). These forests grow on acidic, nutrient-poor, sandy soils derived from Oligo-Miocene sandstone and sometimes they are interspersed with clayish soils derived from layers of marl sediments. Vegetation in the overstory is dominated by the evergreen *Quercus suber* L. In drier lowlands with clayish soils, *Q. suber* trees form open woodlands with the evergreen and shade-intolerant *Olea europaea* var. *sylvestris* Brot. In moister habitats, *Q. suber* coexists with the deciduous shade-tolerant *Quercus canariensis* Willd. forming closed forests (Ojeda *et al.*, 2000). In these forests, *Q. suber* is extensively managed for cork production. Moreover, the adult trees of the species show important problems of defoliation and mortality. Extremely high abundances of *Phytophthora cinnamomi* have been found in soils of symptomatic *Q. suber* trees, and therefore this

pathogen has been suggested as a main driver of the species decline in the area (Gómez-Aparicio *et al.*, 2012).

Study sites, stand structure and tree mortality data

We selected six 1-ha sites within the Natural Park, covering a gradient of climate and soil properties (Table 1). Three of the sites were situated in open woodlands of *Q. suber* and *O. europaea* and the other three in closed forests dominated by *Q. suber* and *Q. canariensis* (see Appendix S1). All selected sites are affected by *Q. suber* decline, with trees showing different levels of defoliation (Gómez-Aparicio *et al.*, 2012). Topography was kept constant in order to avoid confounding effects of the studied variables. Annual precipitation for each plot was obtained from Ninyerola *et al.* (2005).

In each site we mapped and identified all live and dead trees higher than 1.5 m and with a diameter at breast height (dbh) > 2 cm using a total station Leica TC 407. We measured the dbh of each of the trees mapped (n = 1341 trees). In addition, we evaluated the health status of *Q. suber* trees by a visual estimation of crown defoliation on a standardized semi-quantitative scale widely used in the region to monitor oak decline (García *et al.*, 2011; Gómez-Aparicio *et al.*, 2012; Ibáñez *et al.*, 2015): healthy reference trees, slightly defoliated trees (<50% crown defoliation); highly defoliated trees (>50% crown defoliation) and dead trees. Tree conditions were evaluated twice (early spring 2010 and 2012) to ensure healthy status of sampled trees. We found no symptoms of defoliation or mortality in individuals of the coexistent tree species *Q. canariensis* or *O. europaea* in our studied plots.

Wood samples, secondary growth and intrinsic water-use efficiency

Sampling was conducted in autumn 2013. At each of the six study sites we randomly selected five healthy *Q. suber* trees, five defoliated *Q. suber* trees (i.e. > 50% of crown defoliation) and five trees of the coexistent species (*O. europaea* in woodlands or *Q. canariensis* in closed forests) with average size in terms of dbh, height and canopy diameter. For each tree we sampled three branches with similar age, based on the branch diameter (aprox. 5 cm, 14 years), south-exposed and located 4-6 m from the ground (n = 270 sampled branches).

All sampled branches were analyzed for estimating secondary growth and intrinsic water-use efficiency (iWUE). We cut transversely three 1-mm thick sections of each

branch and the samples were air-dried. One section per branch was polished with a series of successively finer sand-paper grits until rings were clearly visible. We scanned those sections at high resolution (600 dpi) and used imageJ v1.49 (Schneider *et al.*, 2012) to measure the total number of annual growth rings and the width of each ring (see Appendix S2). We analyzed secondary growth of branches to avoid the bias from the tree inner rings when decline might have not started yet. We used the other two sections of each branch to analyze intrinsic water-use efficiency (iWUE) from $^{13}C/^{12}C$ isotope ratios in the branch wood. Wood samples were carefully homogenized and milled using a ball-mill (MM301, Retsch. Germany). Aliquots of 0.5-0.7 mg from each wood section were weighed on a balance (AD6 Autobalance Controller, Perkin Elmer., USA) and placed into a tin capsule for isotopic analyses. Cellulose was not extracted, since both whole wood and cellulose isotope show similar trends related to atmospheric CO_2 concentration and climate (Saurer *et al.*, 2004).

The isotope ratio $^{13}C/^{12}C$ was determined on a stable isotope mass spectrometer (ThermoFinnigan MAT 251, CA) at the Stable Isotope Facility at the University of California, Davis. Isotopic values were expressed relative to the international Vienna Pee-Dee Belemnite (V-PDB) standard as $\delta^{13}C$. Two analytical standards were included for analysis after every 10 wood samples: cellulose ($\delta^{13}C$ = -24.72‰) and phthalic acid ($\delta^{13}C$ = -30.63‰). The repeated analysis of these two internal standards yielded a standard deviation <0.1‰ and the accuracy of analyses was 0.07‰. The estimated precision of the measurements was ±0.1‰.

Isotopic discrimination between the C of atmospheric CO_2 and wood carbon (Δ) in plants is a consequence of the preferential fixation of the ^{12}C compared with ^{13}C by plants C_3 during photosynthesis and it was defined by Farquhar and Richards (1984) as:

$$\Delta = \frac{(\delta^{13}C_{atm} - \delta^{13}C_{plant})}{(1 + \delta^{13}C_{plant}/1000)} \quad (1)$$

where $\delta^{13}C_{atm}$ is the isotope ratio of C ($^{13}C/^{12}C$) in atmospheric CO_2 and $\delta^{13}C_{plant}$ is the isotope ratios of C in tree-ring wood, in both cases relative to the standard V-PDB and expressed in parts per thousand (‰). In turn, Δ is linearly related to the ratio of intercellular to atmospheric (Ci/Ca) CO_2 mole fractions (Farquhar *et al.*, 1982):

$$\Delta = a + (b - a)Ci/Ca \quad (2)$$

where a is the fractionation caused by CO_2 diffusion through the stomata (4.4‰), and b is the fractionation caused by Rubisco and PEP carboxylase (27‰; Farquhar &

Richards, 1984). The values for Ca and $\delta^{13}C$ were obtained from McCarroll and Loader (2004). The linear relationship between (Ci/Ca) and Δ can be used to calculate the intrinsic water-use efficiency (iWUE), defined as the ratio of net assimilation to stomatal conductance to water vapor, which can be calculated as:

$$\text{iWUE}=\frac{Ca(b-\Delta)}{1.6(b-a)} \quad (3)$$

The iWUE, expressed in μmol mol^{-1}, has been widely related to trends in the internal regulation of carbon uptake and water loss in plants and is considered the main indirect parameter to integrate over time the instantaneous water use efficiency (Farquhar & Richards, 1984). More details on the calculation of iWUE from $\delta^{13}C$ can be found in McCarroll and Loader (2004).

Soil variables and pathogen analysis

We analyzed abiotic and biotic soil variables under trees in order to relate them to tree water use efficiency and secondary growth. We selected abiotic soil variables related to soil water balance and soil productivity such as soil water potential, texture, depth and organic matter. We analyzed *P. cinnamomi* abundance as a biotic factor because this pathogen is the main driver of the oak decline in these forests (Gómez-Aparicio *et al.*, 2012). We took three soil samples (0-20 cm) under the canopy of each studied tree with a cylindrical auger and mixed to produce one composite soil sample per tree. Soil samples were transported in polyethylene bags in coolers to the laboratory. Total soil depth was recorded under each tree with a metal stick from the surface to the beginning of the bedrock and was calculated as the mean of three measurements under each individual.

Soil samples were air dried at room temperature and sieved at 2 mm to remove root material and stones. A complete particle size analysis was carried out using the Bouyoucos hydrometer method (Gee & Bauder, 1986). From this method we selected sand (0.0625mm < sand < 2mm) as a representative measurement of soil texture. Previously, a subsample of 20 g was used to determine the water content gravimetrically by weighing the fresh and dried soil (105ºC). A subsample of 2 g was then incinerated for 4 h at 550ºC to determine the soil organic matter content by calcinations method (Sparks *et al.*, 1996). Soil water potential was determined by empirical relationships among soil water content and soil texture and organic matter (Saxton *et al.*, 1986; Rawls *et al.*, 2003).

We determined the abundance of *P. cinnamomi* following the method developed by Romero *et al.* (2007). Soil suspensions were prepared by adding 10 g of dry sieved soil in 100 ml of sterilized Water-Agar (0.2%), and aliquots of 1 ml were plated on Petri dishes, each containing 20 ml of the selective medium NARPH (Hüberli *et al.*, 2000). For each soil sample, a total of 20 Petri dishes were prepared. Dishes were incubated at 24ºC in the dark for 24h, then the agar surface of each plate was washed with sterile water and dishes were re-incubated at 24ºC in the dark for 48h. Colonies obtained were identified (using an inverted microscope) by the clustered hyphal swellings typical of *P. cinnamomi* (Erwin & Ribeiro, 1996; Sánchez *et al.*, 2002) and counted. As soil samples were previously dried, it was assumed that each colony obtained resulted from the germination of, at least, one resistant spore (oospore or chlamydospore). Results were expressed as colony forming units per gram of dry soil (CFU g^{-1}).

Data analysis

To test our first hypothesis, we fitted Linear Mixed Models that compare water use efficiency and secondary branch growth of healthy vs. defoliated *Q. suber* trees. We included branch diameter as a covariate in the model for secondary branch growth. To answer our second hypothesis at local scale, we fitted Linear Mixed Models that explain water use efficiency and secondary growth of *Q. suber* trees as a function of abiotic and biotic microsite conditions (i.e. soil depth, soil water potential, sand content, soil organic matter content and *P. cinnamomi* abundance). We fitted separate models for healthy and defoliated trees. The saturated model was successively simplified until the minimal adequate model using Akaike Information Criterion corrected for small samples (AICc, Burnham and Anderson, 2002). Models with a difference in $AIC_c < 2$ units are considered to have equivalent empirical support. We introduced plot as a random factor in all Linear Mixed Models.

To test our third hypothesis, we carried out Linear Mixed Models that compare physiological variables of trees and edaphoclimatic variables between forest types. In order to compare the physiological performance of trees of different species, we fitted Linear Mixed Models including species as a fixed factor (healthy *Q. suber* trees and coexistent *O. europaea* or *Q. canariensis*). As coexistent species differed between forest types, we run the models for all trees but separating *Q. suber* trees in two categories (*Q. suber* in woodlands or *Q. suber* in closed forests). We included branch diameter as a covariate in the model for secondary branch growth. Plot effect was used as a random

factor except for annual precipitation. For this variable we carried out a General Linear Model due to the lack of variability within plots.

All models were fitted using the *nlme* library of the R statistical software v. 3.1.1 (R Core Team, 2014). Parameters of the best model were estimated with restricted maximum likelihood (REML, Zuur *et al.*, 2009) and model selection was carried out using MuMIn package (Bartoń, 2016). Tuckey's tests were applied for multiple comparisons between all pairs of means when significant variables were observed in the LMM. Graphics were performed using R statistical software and Sigmaplot v. 12 (Systat Software Inc.).

Results

Our six study sites were severely affected by decline. The proportion of defoliated *Q. suber* trees varied from 14% to 64%, while the proportion of dead *Q. suber* trees varied from 5% to 38% (Table 1). In all studied forests, we found the presence of *Phytophthora cinnamomi* resting spores in soil samples. The average abundance of this pathogen per plot ranged from 4.63±1.77 $CFU \cdot g^{-1}$ to 440.53±60.65 $CFU \cdot g^{-1}$ (Table 1), reaching densities as high as 1476 $CFU \cdot g^{-1}$ under some defoliated trees. It was found higher pathogen abundance under defoliated than under healthy *Q. suber* trees (LMM $F = 4.65$, $P = 0.036$).

The linear mixed models did not show any difference between defoliated and healthy *Q. suber* trees either in terms of water use efficiency (LMM $F = 0.086$, $P = 0.771$, Fig. 1a) or growth (LMM $F = 0.187$, $P = 0.667$, Fig. 1b). At the local scale, we found that variables explaining variance of water use efficiency and secondary growth differed between healthy and defoliated *Q. suber* trees. The best model of water use efficiency for healthy trees included a negative effect of soil depth, whereas for defoliated trees it contained a negative effect of soil depth and a positive effect of soil pathogen abundance (Table 2, Fig. 2). Regarding secondary branch growth, the null model was the best fit for

Table 1 Structural and edaphoclimatic attributes of the studied sites. Coexistent species are *O. europaea* in woodlands or *Q. canariensis* in closed forests. Defoliated and dead *Q. suber* is expressed as percentage of total *Q. suber* trees. Relative *Q. suber* basal area is expressed as percentage of total basal area of *Q. suber* trees. Data of soil properties and *P. cinnamomi* abundance are mean and standard error ($N = 30$). Ca, K and N analyses and data from Ibáñez *et al.* (2015a).

Variable	WOODLANDS			CLOSED FORESTS		
	S1	S2	S3	S1	S2	S3
Latitude (N)	36º 04′ 38″	36º 18' 37"	36º 31' 69"	36º 06' 09"	36º 23' 10"	36º 28' 13"
Longitude (W)	05º 33' 05"	05º 41' 14"	05º 38' 08"	05º 30' 53"	05º 31' 52"	05º 35' 31"
Forest structure						
Coexistent species stand density (ind·ha^{-1})	48	49	53	277	36	99
Q. suber stand density (ind·ha^{-1})	69	200	265	331	93	78
Defoliated *Q. suber* (%)	36.23	14.00	63.77	26.59	39.78	26.92
Dead *Q. suber* (%)	37.68	7.00	5.28	33.23	15.05	14.10
Relative *Q. suber* basal area (%)						
Healthy	29.51	74.37	26.23	61.05	33.97	39.30
Defoliated	65.84	19.64	37.56	36.44	41.38	32.32
Dead	4.65	5.99	36.21	2.51	24.66	28.39
Site characteristics						
Precipitation (mm)	948.9	726.4	973.1	1067.1	1022.6	1097.0
Soil depth (cm)	35.82 ± 3.03	41.53 ± 3.50	45.02 ± 2.52	49.08 ± 2.27	57.24 ± 2.45	55.82 ± 2.35
Sand (%)	47.06 ± 1.75	68.60 ± 0.99	50.20 ± 1.66	72.90 ± 0.99	68.52 ± 1.04	75.26 ± 0.95
Silt (%)	22.01 ± 0.70	16.29 ± 0.49	17.92 ± 0.40	13.29 ± 0.44	17.78 ± 0.55	14.97 ± 0.53
Clay (%)	30.93 ± 1.30	15.12 ± 0.65	31.88 ± 1.51	13.81 ± 0.69	13.70 ± 0.68	9.77 ± 0.55
Ca (mg kg^{-1})	3397 ± 151	1455 ± 119	5181 ± 348	703 ± 39	961 ± 67	896 ± 57
K (mg kg^{-1})	261.6 ± 14.6	233.4 ± 10.4	299.5 ± 15.6	116.1 ± 6.3	154.5 ± 8.0	183.7 ± 11.4
N (mg g^{-1})	4.84 ± 0.30	3.53 ± 0.19	4.76 ± 0.27	2.49 ± 0.12	2.25 ± 0.14	2.27 ± 0.15
pH	5.94 ± 0.04	5.13 ± 0.03	5.92 ± 0.02	4.89 ± 0.05	5.04 ± 0.04	5.09 ± 0.04
P. cinnamomi abundance (CFU g^{-1})	440.5 ± 60.65	93.83 ± 33.36	355.87±47.36	4.83 ± 1.50	80.17 ± 31.32	4.63 ± 1.77

healthy trees indicating that there was no detectable effect of the studied soil properties on this variable (Appendix S3). For defoliated trees, the best model of secondary growth included a positive effect of soil pathogen abundance (Table 2). This relationship did not have a strong empirical support though, since the difference in AIC with the null model was never larger than two units (see Appendix S3).

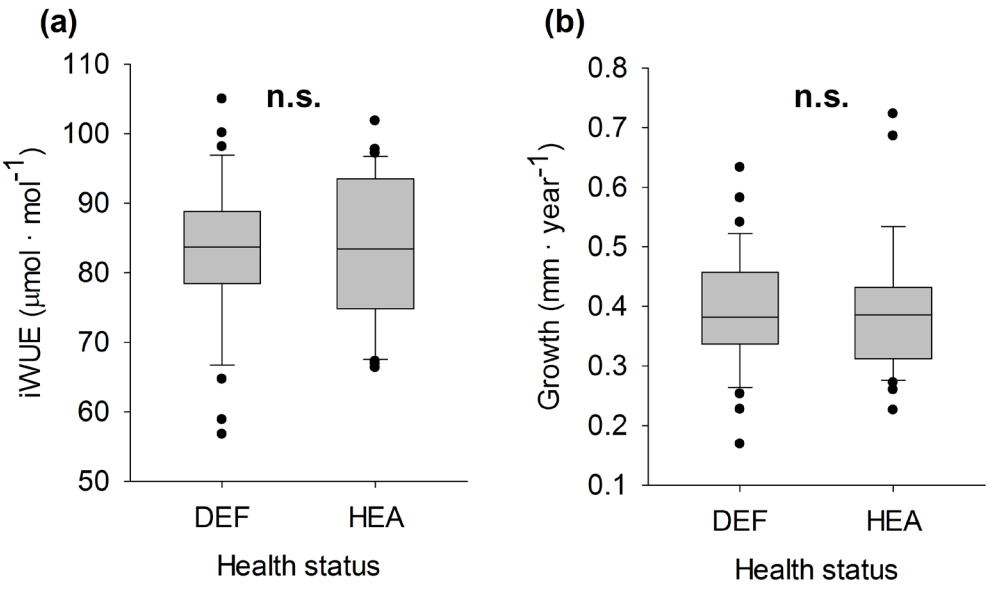

Figure 1 Water use efficiency (a) and secondary branch growth (b) of healthy (HEA) and defoliated *Q. suber* trees (DEF). $N = 30$. n.s.= Non-significant ($P > 0.05$). Each boxplot shows the median, inter-quartiles and whiskers (i.e. 1.5 times inter-quartile rank). Dots outsides the whiskers are outliers.

Table 2 Linear mixed models for analysis of the water use efficiency (WUE) and secondary branch growth of healthy and defoliated *Q. suber*. Degrees of freedom (d.f.), F-ratios and P values of the best models are shown. For growth of healthy *Q. suber*, the best model was the null model (Appendix S3).

Variable	Tree category	Fixed factors	d.f.	*F*	*P*
WUE	Healthy	Depth	1	5.250	0.031
	Defoliated	*P. cinnamomi* abundance	1	13.411	0.001
		Depth	1	4.087	0.055
Growth	Healthy	NULL			
	Defoliated	*P. cinnamomi* abundance	1	11.733	0.002

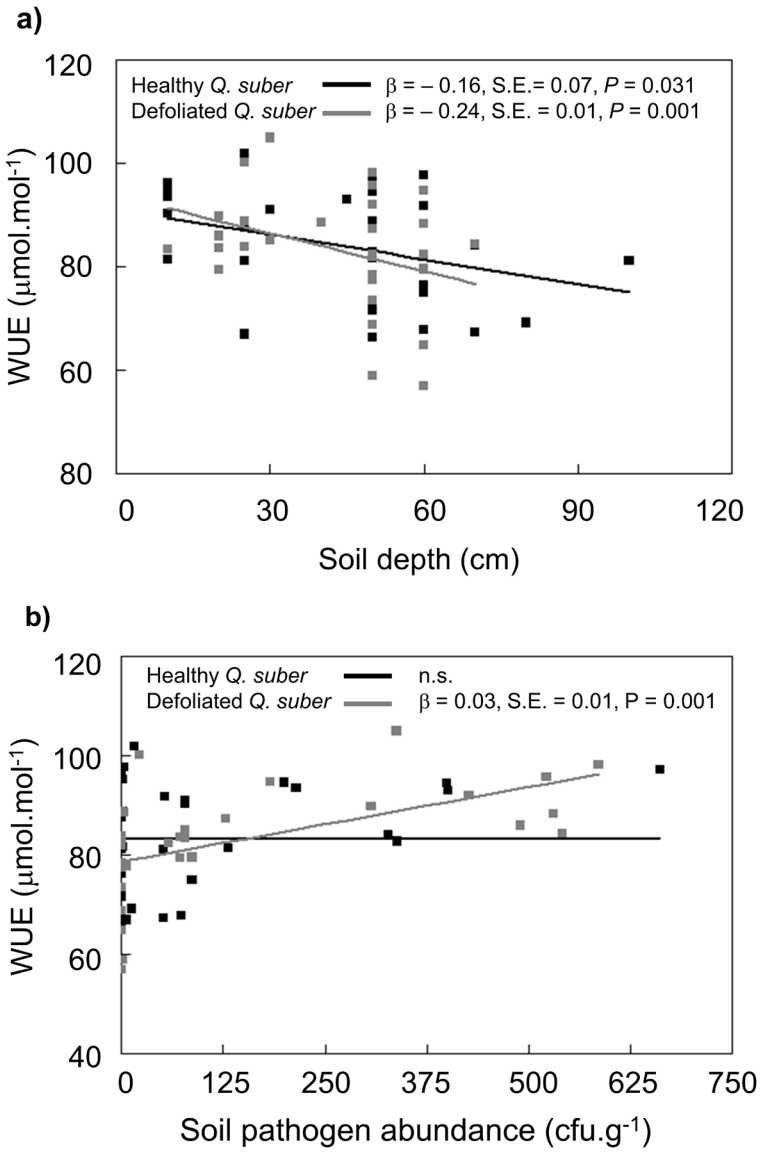

Figure 2 Relationships between water use efficiency of healthy (HEA) and defoliated (DEF) *Q. suber* trees and soil depth (a); and soil pathogen abundance (b). Grey dots and lines are for defoliated *Q. suber* trees and black dots and lines are for healthy *Q. suber* trees. $N = 15$. Estimate (β), standard error (S.E.) and P value of the slopes are shown. n.s.= Non-significant ($P > 0.05$).

At the landscape scale, water use efficiency of *Q. suber* trees differed between forest types, with higher values in woodlands than in closed forests. *Q. suber* trees in woodlands had a 20% higher water use efficiency than *Q. suber* in closed forests (Table 3, Fig. 3a). However secondary branch growth of *Q. suber* trees was similar between forest types (Table 3, Fig. 3b). We also found marginally significant differences between forest types in terms of organic matter, texture and soil pathogen abundance (P<0.10, Table 3, Fig. 3c,d,e), Woodlands had higher soil organic matter but lower sand content than closed forests (Fig. 3c,d). The abundance of *P. cinnamomi* was higher in woodlands than in closed forests (Fig. 3e). Moreover, there was difference in terms of rainfall between forest types, with lower precipitation in woodlands than in closed forests (LM $F_{1,10}$= 12.049, P = 0.006, Fig. 3f). We found differences among *Q. suber* and coexistent non-affected species in terms of water use efficiency (LMM F = 27.030, P < 0.0001, Fig. 4a). We found that *Q. suber* had similar water use efficiency than *O. europaea* in woodlands (P = 0.944), and similar values than *Q. canariensis* in closed forests (P = 0.199). *Q. suber* in woodlands had a 20% higher water use efficiency than what they had in closed forests (P < 0.001, Fig. 4a). We did not find differences in the secondary branch growth among species in both forest types (LMM F = 1.884, P = 0.144, Fig. 4b).

Table 3 Linear mixed models for analysis of the differences between forest types (woodland and closed forest) in water use efficiency and secondary branch growth of *Q. suber* trees, soil organic matter, sand content and *P. cinnamomi* abundance. Degrees of freedom (d.f.), F-ratios and P values of the best models are shown.

Variable	d.f.	*F*	*P*
Water use efficiency	1	52.035	0.002
Secondary branch growth	1	0.003	0.960
Soil organic matter	1	3.969	0.081
Sand content	1	6.489	0.064
P. cinnamomi abundance	1	5.310	0.083

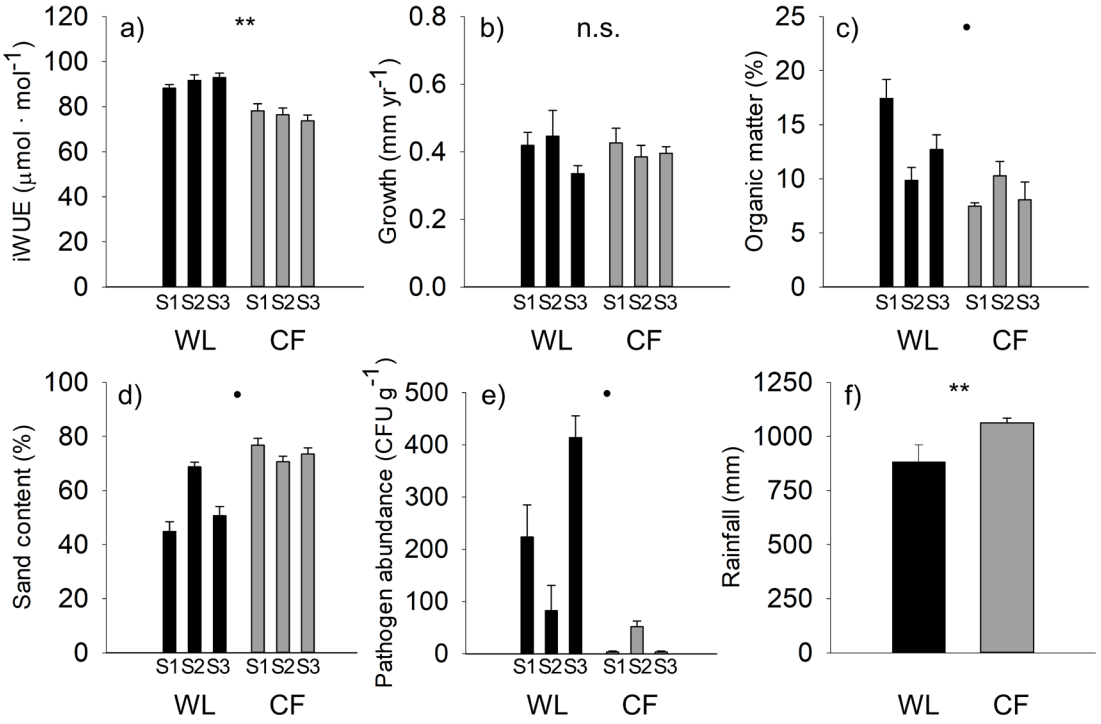

Figure 3 Mean values (±standard error) of water use efficiency (a), secondary branch growth (b), organic matter (c), sand content (d), soil pathogen abundance (e) and precipitation (f) in the three sites (S1, S2, S3) within the two forest types (WL = Wodlands; CF = Closed forests). $N = 10$ for all figures except for (f) where $N = 3$. Significant differences between forest types were shown: point (•) indicates $P<0.10$, single asterisk (*) indicates $P<0.05$; double asterisks (**) indicates $P<0.01$; n.s. indicates $P>0.10$.

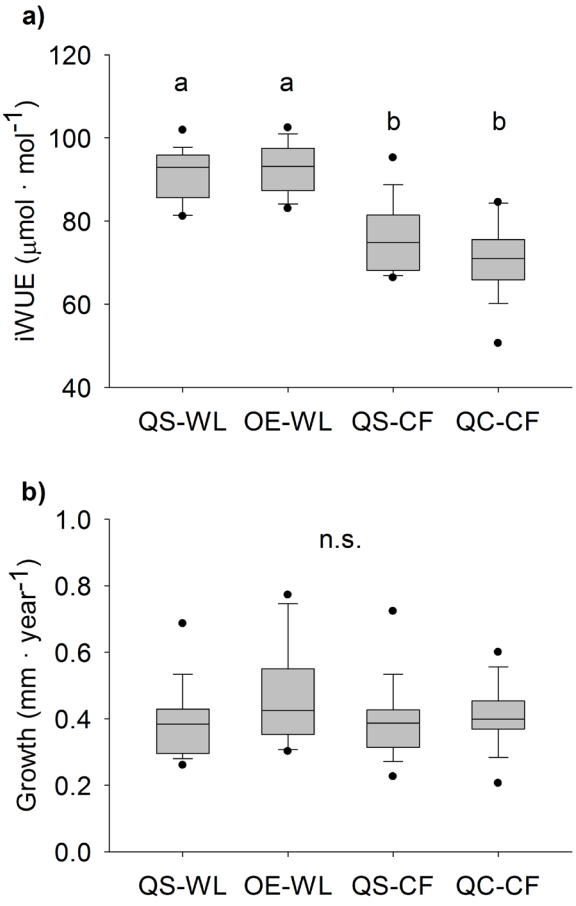

Figure 4 Water use efficiency (iWUE) (a) and secondary branch growth (b) of healthy *Q. suber* trees in each forest type (woodland QS-WL, closed forest QS-CF) and the coexistent species *O. europaea* (OE-WL) and *Q. canariensis* (QC-CF). N = 15. The lower case indicates significant differences in water use efficiency among the tree categories (P<0.05). n.s.= Non-significant (P>0.05).

Discussion

Our results illustrate complex relationships between spatial patterning and coupled ecological processes that help us to understand the phenomenon of *Q. suber* decline. At the whole-tree scale, we did not find that the process of pathogen-induced oak decline led to a straightforward response in water use efficiency and secondary branch growth, contradicting our first hypothesis (Fig. 1). Therefore, our results suggest a decoupling between whole-tree scale defoliation symptoms and the physiological response of trees.

We expected that defoliated trees under stress would show a physiological response to oak decline coupled with defoliation symptoms since trees in water-limited ecosystems have many adaptive mechanisms to cope with drought and biotic disturbances (Peñuelas

et al., 2000). Trees usually respond to drought-induced water stress by closing their stomata to limit water loss and increasing water use efficiency (Tenhunen *et al.*, 1990; Serrano & Peñuelas, 2005). This is a mechanism also expected in trees affected by root pathogens (Fleischmann *et al.*, 2002; Camilo-Alves *et al.*, 2013). However, the reduction in stomatal conductance also increases the risk of carbon starvation (Martínez-Vilalta *et al.*, 2002) together with the risk of hydraulic failure enhanced by a reduced water transport capacity (McDowell *et al.*, 2008; McDowell, 2011). Both mechanisms may induce tree defoliation and mortality (McDowell, 2011; Allen *et al.*, 2015). Although trees native to water-limited ecosystems are able to respond to drought, their physiological phenotypic plasticity and adaptive capacity may be exceeded under intense climate stress in combination with biotic disturbances (Galiano *et al.*, 2012; Sangüesa-Barreda *et al.*, 2013). We also expected lower secondary branch growth in defoliated trees than in healthy trees, as a reduction in tree growth has been previously reported for other evergreen Mediterranean oaks affected by decline (Solla *et al.*, 2009). However, we found that branches of healthy and defoliated trees can growth at similar rate, disregarding the general health status of the tree. It is possible that other variables related with vegetative and reproductive growth, such as trunk growth or seed production, would have better reflected the differences between healthy and defoliated trees. Our results showed that *Q. suber* trees might be unable to mitigate the negative effects of a decrease in water availability related to *P. cinnamomi*-induced decline through a reduction in stomatal conductance, resulting in defoliation and tree death.

At local scale, low soil depth and high pathogen abundance induced trees to stressful situations that led to higher water use efficiency (Fig. 2), which confirms our second hypothesis. Shallow soils can inhibit root growth into deep layers (Fisher & Binkley, 2000), which in turn can limit the access to belowground water resources, especially during summer drought (Costa *et al.*, 2008). Therefore, it should be expected that water use efficiency increases as soil depth decreases, as a consequence of a more efficient stomatal control of water loss by transpiration in shallow soils experimenting water shortage (Tenhunen *et al.*, 1990; Ogaya & Penuelas, 2003; Maseyk *et al.*, 2011). We also detected increasing water use efficiency in defoliated trees as soil pathogen abundance increased. It has been shown that *P. cinnamomi* causes a reduction in fine root abundance in defoliated oaks (Corcobado *et al.*, 2013), which might decrease their water absorption capacity affecting stomatal conductance and increasing water use efficiency (Osswald *et al.*, 2014). For example, previous studies have found physiological responses of *Quercus*

seedlings infected by *P. cinnamomi*, such as stomatal closure, and a more efficient water use and photochemical performance (Luque *et al.*, 1999; Maurel *et al.*, 2001; Robin *et al.*, 2001; Sghaier-Hammami *et al.*, 2013). This response may enable trees to tolerate water stress. However, although we detected an ecophysiological response of defoliated *Q. suber* trees to high pathogen loads, we did not find that those trees had on average higher water use efficiency than healthy *Q. suber* trees. Our results suggest that *Q. suber* trees subjected to soil drought and root pathogens increase water use efficiency to some extent, while this response might not be enough to overcome the physiological stress eventually leading to defoliation and death.

Results obtained at local scale seem to agree with patterns obtained at a landscape scale. Water use efficiency differed significantly between open woodlands and closed forests, as we hypothesized (Fig. 3). Open woodlands were characterized by lower precipitation but also their soils had lower sand and higher organic matter contents than closed forests. These soil differences are usually related to milder effects of water shortage on plants (Sperry & Hacke, 2002; Galiano *et al.*, 2010). However, closed forests were characterized by higher precipitation and frequent fogs, even during the summer dry season, which can reduce significantly the water stress of trees (Ojeda *et al.*, 2000; Urbieta *et al.*, 2008) and explain the lower water use efficiency of trees in this forest type (see Appendix S1). It has been shown that changes in external drivers or disturbances can alter pattern-process relationships (Allen, 2007; Peters *et al.*, 2007). For example, we could expect that the links between water stress and water use efficiency would be altered as a consequence of the introduction of *P. cinnamomi*. Despite new processes and feedbacks might be observed as a result of the oak decline, our results did not suggest any change in the relationship between water stress and the water use efficiency at local and landscape scales.

Our findings showed at landscape scale a high plasticity of *Q. suber* to modify its water use efficiency depending on the forest type where trees grow (Fig. 4). The large range of values observed in carbon isotope discrimination and the differences in this variable between forest types seems to suggest that *Q. suber* trees can adjust efficiently the stomatal conductance in relation to local water availability (Otieno *et al.*, 2007; Gouveia & Freitas, 2009). The water use efficiency variance obtained here is within the range of those reported for other tree species with high plasticity in response to water availability (Ferrio *et al.*, 2003; Shestakova *et al.*, 2014). In our study we obtained that *Q. suber* modified their water use efficiency up to similar values of the semi-deciduous *Q.*

canariensis growing in closed forests. This convergent response can be explained as evergreen and deciduous *Quercus* species may show similar responses to water constraint in terms of stomatal conductance and net CO_2 assimilation under mesic conditions (Damesin *et al.*, 1998). However, *Q. suber* was able to increase even more its water use efficiency, reaching in open woodlands similar values of than *O. europaea*, a species with anatomical and hydraulic traits that enable high drought stress tolerance (Rossi *et al.*, 2013). Our results support that *Q. suber* trees are able to increase water use efficiency in drier sites while they keep growth rates similar to those found in wetter sites, at least in terms of branch secondary growth.

Conclusions

Oak decline from Mediterranean forest ecosystems is considered an important concern for managers, landowners and researchers because in the last decades a decrease in vigor and an increase in mortality of evergreen oak trees threaten these forests, and consequently, the goods and services these systems provide. Our results suggest that, although *Q. suber* is able to respond ecophysiologically to water stress at local and landscape scale, the overall response of defoliated trees is not enough to overcome the stress induced by *P. cinnamomi*. This fact might have been responsible of high mortality rates when *Q. suber* forests are affected by global change drivers such as drought and invasive pathogens. Particularly in our study area, more than the half of the basal area of *Q. suber* was affected by defoliation and high tree mortality rates. A lack of coevolutionary history between *Q. suber* and *P. cinnamomi* is probably causing the high vulnerability of this tree species to the pathogen. Overall, our results illustrate the complex way by which adult *Q. suber* trees physiologically respond to *P. cinnamomi*-induced mortality and may help to anticipate the subsequent consequences if the oak decline becomes widespread in the future.

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References

Allen CD. 2007. Interactions across spatial scales among forest dieback, fire, and erosion in northern New Mexico landscapes. Ecosystems 10(5): 797-808.

Allen CD, Breshears DD, McDowell NG. 2015. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere 6(8): art129.

Anderegg WRL, Kane JM, Anderegg LDL. 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. Nature Climate Change 3(1): 30-36.

Ávila JM, Gallardo A, Ibáñez B, Gómez-Aparicio L. 2016. Quercus suber dieback alters soil respiration and nutrient availability in Mediterranean forests. Journal of Ecology 104(5): 1441-1452.

Balci Y, Long RP, Mansfield M, Balser D, MacDonald WL. 2010. Involvement of Phytophthora species in white oak (Quercus alba) decline in southern Ohio. Forest Pathology 40(5): 430-442.

Bartoń K 2016. MuMIn: multi-model inference. R package version.

Bendixsen DP, Hallgren SW, Frazier AE. 2015. Stress factors associated with forest decline in xeric oak forests of south-central United States. Forest Ecology and Management 347: 40-48.

Brasier CM. 1992. Oak tree mortality in Iberia. Nature 360(6404): 539.

Brasier CM. 1996. Phytophthora cinnamomi and oak decline in southern Europe. Environmental constraints including climate change. Annals of Forest Science 53(2-3): 347-358.

Brouwers NC, Mercer J, Lyons T, Poot P, Veneklaas E, Hardy G. 2013. Climate and landscape drivers of tree decline in a Mediterranean ecoregion. Ecology and Evolution 3(1): 67-79.

Camilo-Alves CSP, da Clara MIE, Ribeiro NA. 2013. Decline of Mediterranean oak trees and its association with Phytophthora cinnamomi: a review. European Journal of Forest Research 132(3): 411-432.

Corcobado T, Cubera E, Juárez E, Moreno G, Solla A. 2014. Drought events determine performance of Quercus ilex seedlings and increase their susceptibility to Phytophthora cinnamomi. Agricultural and Forest Meteorology 192-193: 1-8.

Corcobado T, Cubera E, Moreno G, Solla A. 2013. Quercus ilex forests are influenced by annual variations in water table, soil water deficit and fine root loss caused by Phytophthora cinnamomi. Agricultural and Forest Meteorology 169: 92-99.

Corcobado T, Solla A, Madeira M, Moreno G. 2013. Combined effects of soil properties and Phytophthora cinnamomi infections on Quercus ilex decline. Plant and Soil 373(1-2): 403-413.

Costa A, Madeira M, Oliveira ÂC. 2008. The relationship between cork oak growth patterns and soil, slope and drainage in a cork oak woodland in Southern Portugal. Forest Ecology and Management 255(5): 1525-1535.

Costa A, Pereira H, Madeira M. 2010. Analysis of spatial patterns of oak decline in cork oak woodlands in Mediterranean conditions. Annals of Forest Science 67(2): 204p201-204p210.

Chave J. 2013. The problem of pattern and scale in ecology: what have we learned in 20years? Ecology Letters 16: 4-16.

Damesin C, Rambal S, Joffre R. 1998. Co-occurrence of trees with different leaf habit: a functional approach on Mediterranean oaks. Acta Oecologica 19(3): 195-204.

Edburg SL, Hicke JA, Brooks PD, Pendall EG, Ewers BE, Norton U, Gochis D, Gutmann ED, Meddens AJH. 2012. Cascading impacts of bark beetle-caused tree mortality on coupled biogeophysical and biogeochemical processes. Frontiers in Ecology and the Environment 10(8): 416-424.

Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppel BD, Knoepp JD, Lovett GM, Mohan J, Orwig DA, Rodenhouse NL, Sobczak WV, Stinson KA, Stone JK, Swan CM, Thompson J, Von Holle B, Webster JR. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment 3(9): 479-486.

Erwin DC, Ribeiro OK. 1996. Phytophthora Diseases Worldwide. St Paul, Minnesota: APS Press.

Farquhar G, O'Leary M, Berry J. 1982. On the Relationship Between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. Functional Plant Biology 9(2): 121-137.

Farquhar G, Richards R. 1984. Isotopic Composition of Plant Carbon Correlates With Water-Use Efficiency of Wheat Genotypes. Functional Plant Biology 11(6): 539-552.

Ferrio JP, Florit A, Vega A, Serrano L, Voltas J. 2003. Δ13C and tree-ring width reflect different drought responses in Quercus ilex and Pinus halepensis. Oecologia 137(4): 512-518.

Fisher R, Binkley D. 2000. Ecology and management of forest soils. NY, USA: John Wiley & Sons.

Fleischmann F, Schneider D, Matyssek R, Oβwald W. 2002. Investigations on net CO2 assimilation, transpiration and root growth of Fagus sylvatica infested with four different Phytophthora species. Plant Biology 4(2): 144-152.

Galiano L, Martínez-Vilalta J, Lloret F. 2010. Drought-Induced Multifactor Decline of Scots Pine in the Pyrenees and Potential Vegetation Change by the Expansion of Co-occurring Oak Species. Ecosystems 13(7): 978-991.

Galiano L, Martinez-Vilalta J, Sabate S, Lloret F. 2012. Determinants of drought effects on crown condition and their relationship with depletion of carbon reserves in a Mediterranean holm oak forest. Tree Physiology 32(4): 478-489.

García LV, Ramo C, Aponte C, Moreno A, Domínguez MT, Gómez-Aparicio L, Redondo R, Marañón T. 2011. Protected wading bird species threaten relict centenarian cork oaks in a Mediterranean Biosphere Reserve: A conservation management conflict. Biological Conservation 144(2): 764-771.

Gee GW, Bauder JW 1986. Particle-size Analysis. In: Klute A ed. Methods of Soil Analysis: Part 1—Physical and Mineralogical Methods. Madison, WI: Soil Science Society of America, American Society of Agronomy.

Gómez-Aparicio L, Ibáñez B, Serrano MS, De Vita P, Ávila JM, Pérez-Ramos IM, García LV, Sánchez ME, Marañón T. 2012. Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration. New Phytologist 194(4): 1014-1024.

Gouveia AC, Freitas H. 2009. Modulation of leaf attributes and water use efficiency in Quercus suber along a rainfall gradient. Trees 23(2): 267-275.

Haavik LJ, Billings SA, Guldin JM, Stephen FM. 2015. Emergent insects, pathogens and drought shape changing patterns in oak decline in North America and Europe. Forest Ecology and Management 354: 190-205.

Hüberli D, Tommerup IC, Hardy GESJ. 2000. False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with Phytophthora cinnamomi. Australasian Plant Pathology 29(3): 164-169.

Ibáñez B, Gómez-Aparicio L, Ávila JM, Pérez-Ramos IM, García LV, Marañón T. 2015a. Impact of tree decline on spatial patterns of seedling-mycorrhiza interactions: Implications for regeneration dynamics in Mediterranean forests. Forest Ecology and Management.

Ibáñez B, Gómez-Aparicio L, Stoll P, Ávila JM, Pérez-Ramos IM, Marañón T. 2015b. A neighborhood analysis of the consequences of Quercus suber decline for regeneration dynamics in Mediterranean forests. PloS one 10(2): e0117827.

Jung T, Orlikowski L, Henricot B, Abad-Campos P, Aday AG, Aguín Casal O, Bakonyi J, Cacciola SO, Cech T, Chavarriaga D, Corcobado T, Cravador A, Decourcelle T, Denton G, Diamandis S, Doğmuş-Lehtijärvi HT, Franceschini A, Ginetti B, Green S, Glavendekić M, Hantula J, Hartmann G, Herrero M, Ivic D, Horta Jung M, Lilja A, Keca N, Kramarets V, Lyubenova A, Machado H, Magnano di San Lio G, Mansilla Vázquez PJ, Marçais B, Matsiakh I, Milenkovic I, Moricca S, Nagy ZÁ, Nechwatal J, Olsson C, Oszako T, Pane A, Paplomatas EJ, Pintos Varela C, Prospero S, Rial Martínez C, Rigling D, Robin C, Rytkönen A, Sánchez ME, Sanz Ros AV, et al. 2016. Widespread Phytophthora infestations in European nurseries put forest, semi-natural and horticultural ecosystems at high risk of Phytophthora diseases. Forest Pathology 46(2): 134-163.

Levin SA. 1992. The problem of pattern and scale in ecology. Ecology 73(6): 1943-1967.

Lowe S, Browne M, Boudjelas S, De Poorter M. 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database.: Invasive Species Specialist Group Species Survival Commission, World Conservation Union (IUCN), Auckland, New Zealand.

Luque J, Cohen M, Save R, Biel C, Alvarez IF. 1999. Effects of three fungal pathogens on water relations, chlorophyll fluorescence and growth of Quercus suber L. Annals of Forest Science 56(1): 19-26.

Lloret F, Escudero A, Iriondo JM, Martínez-Vilalta J, Valladares F. 2012. Extreme climatic events and vegetation: the role of stabilizing processes. Global Change Biology 18(3): 797-805.

Marañón T, Ajbilou R, Ojeda F, Arroyo J. 1999. Biodiversity of woody species in oak woodlands of southern Spain and northern Morocco. Forest Ecology and Management 115(2–3): 147-156.

Marañón T, Ibáñez B, Anaya-Romero M, Muñoz-Rojas M, Pérez-Ramos I 2012. Oak trees and woodlands providing ecosystem services in southern Spain. In: Ian D. Rotherham, Christine Handley, Mauro Agnoletti, Samojlik T eds. Trees Beyond the Wood. Sheffield, UK: Wildtrack Publishing, 369-378.

Martínez-Vilalta J, Prat E, Oliveras I, Piñol J. 2002. Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. Oecologia 133(1): 19-29.

Maseyk K, Hemming D, Angert A, Leavitt SW, Yakir D. 2011. Increase in water-use efficiency and underlying processes in pine forests across a precipitation gradient in the dry Mediterranean region over the past 30 years. Oecologia 167(2): 573-585.

Maurel M, Robin C, Capron G, Desprez-Loustau ML. 2001. Effects of root damage associated with Phytophthora cinnamomi on water relations, biomass accumulation, mineral nutrition and vulnerability to water deficit of five oak and chestnut species. Forest Pathology 31(6): 353-369.

McCarroll D, Loader NJ. 2004. Stable isotopes in tree rings. Quaternary Science Reviews 23(7): 771-801.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist 178(4): 719-739.

McDowell NG. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. Plant Physiology 155(3): 1051-1059.

Ninyerola M, Pons X, Roure JM. 2005. Atlas climático digital de la Península Ibérica: metodología y aplicaciones en bioclimatología y geobotánica. Barcelona: Universidad Autónoma de Barcelona.

Ogaya R, Penuelas J. 2003. Comparative field study of Quercus ilex and Phillyrea latifolia: photosynthetic response to experimental drought conditions. Environmental and Experimental Botany 50(2): 137-148.

Ojeda F, Marañón T, Arroyo J. 2000. Plant diversity patterns in the Aljibe Mountains (S. Spain): A comprehensive account. Biodiversity and Conservation 9(9): 1323-1343.

Olea L, San Miguel-Ayanz A. 2006. The Spanish dehesa. A traditional Mediterranean silvopastoral system linking production and nature conservation. Grassland Science in Europe 11: 3-13.

Osswald W, Fleischmann F, Rigling D, Coelho AC, Cravador A, Diez J, Dalio RJ, Jung MH, Pfanz H, Robin C, Sipos G, Solla A, Cech T, Chambery A, Diamandis S, Hansen E, Jung T, Orlikowski LB, Parke J, Prospero S, Werres S. 2014. Strategies of attack and defence in woody plant-Phytophthora interactions. Forest Pathology 44(3): 169-190.

Otieno D, Schmidt M, Kurz-Besson C, Do Vale RL, Pereira J, Tenhunen J. 2007. Regulation of transpirational water loss in Quercus suber trees in a Mediterranean-type ecosystem. Tree Physiology 27(8): 1179-1187.

Peñuelas J, Filella I, Lloret F, Piñol J, Siscart D. 2000. Effects of a Severe Drought on Water and Nitrogen Use by Quercus ilex and Phyllyrea latifolia. Biologia Plantarum 43(1): 47-53.

Pérez-Sierra A, López-García C, León M, García-Jiménez J, Abad-Campos P, Jung T, Belbahri L. 2013. Previously unrecorded low-temperature Phytophthoras pecies associated with Quercus decline in a Mediterranean forest in eastern Spain. Forest Pathology 43(4): 331-339.

Peters DP, Bestelmeyer BT, Turner MG. 2007. Cross–scale interactions and changing pattern–process relationships: consequences for system dynamics. Ecosystems 10(5): 790-796.

R Core Team 2014. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rawls W, Pachepsky YA, Ritchie J, Sobecki T, Bloodworth H. 2003. Effect of soil organic carbon on soil water retention. Geoderma 116(1): 61-76.

Robin C, Capron G, Desprez-Loustau ML. 2001. Root infection by Phytophthora cinnamomi in seedlings of three oak species. Plant Pathology 50(6): 708-716.

Romero MA, Sanchez JE, Jimenez JJ, Belbahri L, Trapero A, Lefort F, Sanchez ME. 2007. New Pythium taxa causing root rot on Mediterranean Quercus species in South-West Spain and Portugal. Journal of Phytopathology 155(5): 289-295.

Rossi L, Sebastiani L, Tognetti R, d'Andria R, Morelli G, Cherubini P. 2013. Tree-ring wood anatomy and stable isotopes show structural and functional adjustments in olive trees under different water availability. Plant and Soil 372(1-2): 567-579.

Sánchez ME, Caetano P, Ferraz J, Trapero A. 2002. Phytophthora disease of Quercus ilex in south-western Spain. Forest Pathology 32(1): 5-18.

Sangüesa-Barreda G, Linares JC, Julio Camarero J. 2013. Drought and mistletoe reduce growth and water-use efficiency of Scots pine. Forest Ecology and Management 296(0): 64-73.

Saurer M, Siegwolf RT, Schweingruber FH. 2004. Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. Global Change Biology 10(12): 2109-2120.

Saxton K, Rawls WJ, Romberger J, Papendick R. 1986. Estimating generalized soil-water characteristics from texture. Soil Science Society of America Journal 50(4): 1031-1036.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9(7): 671-675.

Serrano L, Peñuelas J. 2005. Contribution of physiological and morphological adjustments to drought resistance in two Mediterranean tree species. Biologia Plantarum 49(4): 551-559.

Sghaier-Hammami B, Valero-Galvan J, Romero-Rodriguez MC, Navarro-Cerrillo RM, Abdelly C, Jorrin-Novo J. 2013. Physiological and proteomics analyses of Holm oak (Quercus ilex subsp ballota Desf. Samp.) responses to Phytophthora cinnamomi. Plant Physiology and Biochemistry 71: 191-202.

Shestakova TA, Aguilera M, Ferrio JP, Gutierrez E, Voltas J. 2014. Unravelling spatiotemporal tree-ring signals in Mediterranean oaks: a variance-covariance modelling approach of carbon and oxygen isotope ratios. Tree Physiology 34(8): 819-838.

Solla A, García L, Pérez A, Cordero A, Cubera E, Moreno G. 2009. Evaluating potassium phosphonate injections for the control of Quercus ilex decline in SW Spain: implications of low soil contamination by Phytophthora cinnamomi and low soil water content on the effectiveness of treatments. Phytoparasitica 37(4): 303-316.

Sparks DL, Page A, Helmke P, Loeppert R, Soltanpour P, Tabatabai M, Johnston C, Sumner M. 1996. Methods of soil analysis. Part 3. Chemical methods: Soil Science Society of America Inc.

Sperry J, Hacke U. 2002. Desert shrub water relations with respect to soil characteristics and plant functional type. Functional Ecology 16(3): 367-378.

Tenhunen J, Serra AS, Harley P, Dougherty R, Reynolds JF. 1990. Factors influencing carbon fixation and water use by Mediterranean sclerophyll shrubs during summer drought. Oecologia 82(3): 381-393.

Thomas F, Blank R, Hartmann G. 2002. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. Forest Pathology 32(4-5): 277-307.

Urbieta IR, Zavala MA, Marañón T. 2008. Human and non-human determinants of forest composition in southern Spain: Evidence of shifts towards cork oak dominance as a result of management over the past century. Journal of Biogeography 35(9): 1688-1700.

van Mantgem PJ, Stephenson NL, Byrne JC, Daniels LD, Franklin JF, Fule PZ, Harmon ME, Larson AJ, Smith JM, Taylor AH, Veblen TT. 2009. Widespread increase of tree mortality rates in the western United States. Science 323(5913): 521-524.

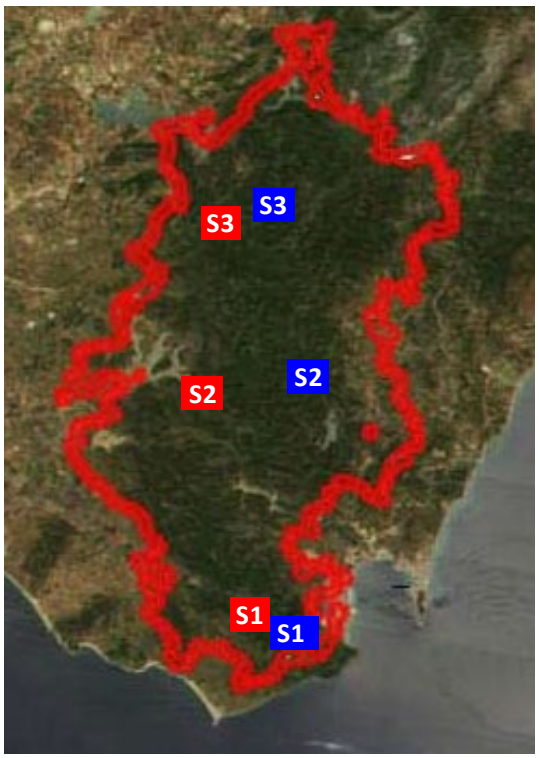
Weste G, Marks GC. 1987. The biology of Phytophthora cinnamomi in australasian forests. Annual Review of Phytopathology 25: 207-229.

Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM. 2009. Mixed Effects Models and Extensions in Ecology with R: Springer.

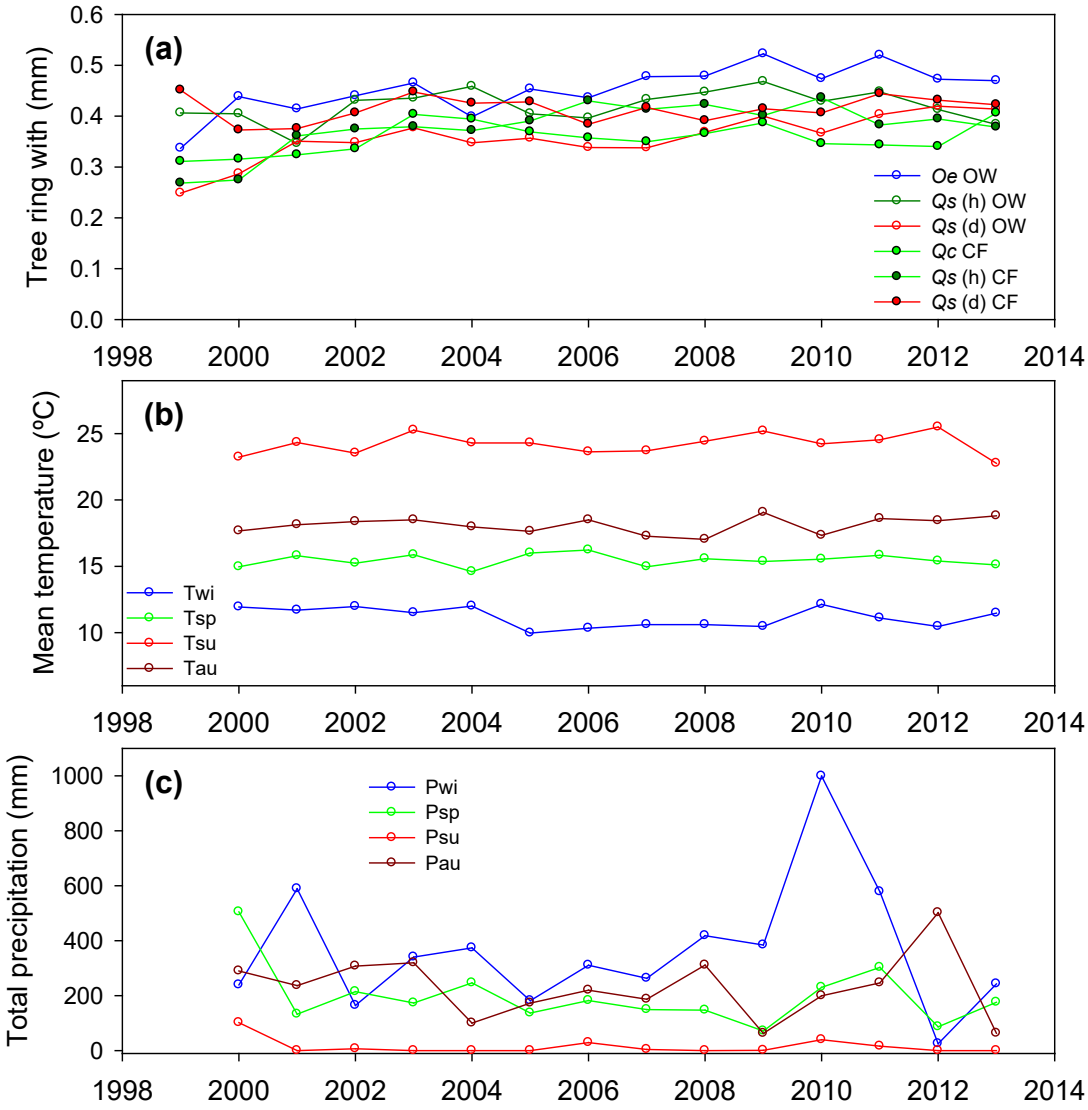
Supporting information

Appendix S1 Location map of Los Alcornocales Natural Park and study sites. Pictures of both forest types (Woodlands and Closed Forests).

Los Alcornocales Natural Park



Appendix S2 Tree ring with (a) obtained in *Olea europaea*, Oe; healty *Quercus suber*, Qs (h); defoliated *Quercus suber*, Qs (d); and *Quercus canariensis*, Qc, trees sampled within open woodland (OW) and closed forests (CF), respectively. Seasonal mean temperature (b) and seasonal total precipitation (c) obtained in the Meteorological Station from Jimena de la Frontera (Location 36º 24' 49" N, 05º, 23' 01" W; 53 m above sea level and located in the center of the Natural Park) for the period 1999-2013. Seasonal temperature (T) and precipitation (P) were computed for winter (wi, december of the previous year, January and February), spring (sp, March, April, May), summer (su, June, July, August), and autumn (au, September, October, November).



Appendix S3. Best-fitting regression models of water use efficiency and secondary branch growth for healthy and defoliated trees. Only the models with AICc lower than the null model (i.e. model that contained only a constant) are presented. Models are ranked according to their AICc value. ΔAICc represents the difference in AICc from the model with the lowest AICc score. The model with lowest value of AICc and thus ΔAICc = 0 represents the model with the strongest empirical support. PCI = *P. cinnamomi* abundance, DEP = Soil depth, SAN = Sand content, SOM = Soil organic matter.

Predicted variable	Tree healht status	Explicative variables	ΔAICc
Water use efficiency	Healthy	DEP	0.00
		PCI+DEP	0.47
		Null	2.46
	Defoliated	PCI+DEP	0.00
		PCI+DEP+SOM	2.92
		PCI+DEP+SAN	2.93
		PCI	3.20
		DEP+SOM	3.99
		DEP+SAN	4.54
		Null	4.84
Secondary growht	Healthy	Null	0.00
	Defoliated	PCI	0.00
		PCI+SAN	0.14
		Null	0.42

CAPÍTULO 3

QUERCUS SUBER DIEBACK ALTERS SOIL RESPIRATION AND NUTRIENT AVAILABILITY IN MEDITERRANEAN FORESTS

Este capítulo reproduce el siguiente manuscrito:
Ávila, J.M., Gallardo, A., Ibáñez, I., Gómez-Aparicio. 2016. *Quercus suber* dieback alters soil respiration and nutrient availability in Mediterranean forests. Journal of Ecology 104, 1441–1452. DOI: 10.1111/1365-2745.12618

Quercus suber dieback alters soil respiration and nutrient availability in Mediterranean forests

Resumen

1. Recientemente se ha detectado un incremento en las tasas de mortalidad en bosques a escala mundial. Sin embargo, pocos trabajos se han centrado en estudiar las consecuencias potenciales que el decaimiento podría tener para el funcionamiento del ecosistema.

2. En este trabajo se ha estudiado el efecto del decaimiento y muerte de *Quercus suber* en los ciclos de carbono, nitrógeno y fósforo en dos tipos de bosques mediterráneos (bosque abierto y bosque cerrado) afectados por el patógeno *Phytophthora cinnamomi*. Se utilizó una aproximación de vecindad espacialmente explícita para analizar los efectos directos del decaimiento de *Q. suber* en las variables del suelo, comparando el impacto de individuos de *Q. suber* con diferente estado de salud, así como su potencial efecto indirecto a largo plazo, al comparar el impacto con el de especies coexistentes no afectadas por decaimiento.

3. El decaimiento de *Q. suber* provocó un descenso en las tasas de respiración del suelo y la disponibilidad de fósforo, mientras que el efecto sobre el nitrógeno varió entre tipos de bosque. El efecto de las especies coexistentes en comparación al efecto de *Q. suber* fue marcadamente diferente respecto a la disponibilidad de nutrientes, pero similar respecto a las tasas de respiración del suelo. Los modelos mostraron una baja variabilidad inter-anual, pero elevada variabilidad intra-anual en los impactos del decaimiento sobre el ecosistema.

4. Síntesis. Los resultados de este trabajo apoyan la idea de que el decaimiento de árboles podría tener un fuerte efecto tanto a corto como a largo plazo en los procesos ecosistémicos de bosques mediterráneos. Con este trabajo se proporciona una valiosa información que permite aumentar la base de conocimiento existente sobre los impactos ecosistémicos tanto del decaimiento en general, como de la mortalidad asociada a *P. cinnamomi* en particular. Debido a que se prevé que tanto la actividad como la distribución de este patógeno aumenten como consecuencia del calentamiento del clima, los impactos negativos asociados al patógeno podrían incrementarse en el futuro inmediato, afectando al funcionamiento de ecosistemas en todo el mundo.

Abstract

1. An increase in tree mortality rates has been recently detected in forests worldwide. However, few works have focused on the potential consequences of forest dieback for ecosystem functioning.

2. Here we assessed the effect of *Quercus suber* dieback on carbon, nitrogen and phosphorus cycles in two types of Mediterranean forests (woodlands and closed forests) affected by the aggressive pathogen *Phytophthora cinnamomi*. We used a spatially-explicit neighbourhood approach to analyse the direct effects of *Q. suber* dieback on soil variables, comparing the impact of *Q. suber* trees with different health status, as well as its potential long-term indirect effects, comparing the impact of non-declining coexistent species.

3. *Quercus suber* dieback translated into lower soil respiration rates and phosphorus availability, whereas its effects on nitrogen varied depending on forest type. Coexistent species differed strongly from *Q. suber* in their effects on nutrient availability, but not on soil respiration rates. Our models showed low inter-annual but high intra-annual variation in the ecosystem impacts of tree dieback.

4. *Synthesis*. Our results support that tree dieback might have important short- and long-term impacts on ecosystem processes in Mediterranean forests. With this work we provide valuable insights to fill the existent gap in knowledge on the ecosystem-level impacts of forest dieback in general and *P. cinnamomi*-driven mortality in particular. Because the activity and range of this pathogen is predicted to increase due to climate warming, these impacts could also increase in the near future altering ecosystem functioning worldwide.

Keywords

Carbon cycle, drought, forest disease, invasive species, neighbourhood, nutrient availability, plant-soil (below-ground) interactions, *Phytophthora cinnamomi*, soil pathogen, tree mortality.

Introduction

In the last decades, an increase in tree mortality rates (forest dieback *sensu* Allen (2009)) has been detected in forests worldwide (e.g. van Mantgem *et al.*, 2009; Allen *et al.*, 2010; Carnicer *et al.*, 2011). Causes underlying this phenomenon are complex and uncertain, but they are frequently associated with global change drivers such as increasing temperatures and droughts, invasive pests and pathogens, or interactions between these factors (Martínez-Vilalta *et al.*, 2012; McDowell *et al.*, 2013). The process of tree defoliation and mortality can have strong direct impacts on ecosystem functioning, causing a reduction in primary productivity and changes in the circulation of nutrients (Lovett *et al.*, 2006; Hicke *et al.*, 2012; Wang *et al.*, 2012). Moreover, increased tree mortality rates could indirectly affect the function of the whole ecosystem in the long term if species replacements occur due to differences among species in their vulnerability to mortality factors (Ellison *et al.*, 2005; Lovett *et al.*, 2010). Despite the potential for large direct and indirect ecosystem-level impacts, our current understanding of the consequences of forest dieback on biogeochemical cycles is still limited (see reviews by Hicke *et al.*, 2012; Wang *et al.*, 2012; Anderegg *et al.*, 2013).

Soil CO_2 fluxes −including autotrophic (root and rhizosphere) and heterotrophic (microbes) respiration− are a key component of the terrestrial carbon cycle that might be dramatically altered by an increase in tree mortality rates (Kurz *et al.*, 2008; Hicke *et al.*, 2012; Anderegg *et al.*, 2013). Tree defoliation and mortality has been frequently found to cause a reduction in soil respiration rates, explained by a decrease in root activity (Nuckolls *et al.*, 2009; Nave *et al.*, 2011). However, other studies have found no changes (Morehouse *et al.*, 2008; Brown *et al.*, 2010) or even increments (Barba *et al.*, 2013; Lloret *et al.*, 2015) in soil respiration rates as a consequence of higher microbial respiration due to post-mortality increases in litter inputs or changes in microclimate. Therefore, the implications of forest dieback for soil respiration are complex and depend on the balance of its effects on root and microbial respiration (e.g. Morehouse *et al.*, 2008; Hicke *et al.*, 2012). Given that forest dieback usually implies a reduction in gross primary productivity - at least in the short-term (i.e. weeks to years following Hicke *et al.*, 2012) - assessing its impact on soil respiration rates is key to foresee potential changes in the role of forests in the terrestrial carbon cycle, which might change from a carbon sink to a carbon source as tree mortality increases (Kurz *et al.*, 2008; Hicke *et al.*, 2012).

Tree defoliation and mortality could also have pervasive effects on nitrogen (N) and phosphorus (P) cycles, two of the most important limiting nutrients in terrestrial ecosystems (e.g. Attiwill & Adams, 1993). However, the number of existing studies addressing the effect of forest dieback on soil nutrients is low, particularly for P (Block *et al.*, 2013). The few N studies available have found mainly increases in soil N after tree mortality due to a reduction in vegetative uptake or changes in litter quantity and quality and its mineralization (e.g. Kizlinski *et al.*, 2002; Morehouse *et al.*, 2008). The case of P is however more complex. P availability might increase due to lower plant uptake after tree death (Block *et al.*, 2013). However, tree mortality also implies a reduction in root phosphatase activity (responsible of the mineralization of organic P, Schneider *et al.*, 2001; George *et al.*, 2011) and in the exudation of organic acids (responsible of the solubilization of mineral P, Jurinak *et al.*, 1986; Hinsinger, 2001). Therefore, the result of a decrease in root exudations (i.e. rhizodeposition) could translate into reductions in P availability underneath declining trees. Understanding the effects of tree mortality on N and P cycles is of paramount importance, since any change in the availability of these limiting nutrients might impact multiple processes after forest dieback such as forest productivity or vegetation recovery (e.g. Mladenoff, 1987; Edburg *et al.*, 2012).

In the long-term (at decade scale following Hicke *et al.*, 2012), the impacts of forest dieback on ecosystem function as a consequence of species replacements during secondary succession might be even more severe than the direct effects of tree defoliation and mortality (Hancock *et al.*, 2008; Lovett *et al.*, 2010; Barba *et al.*, 2013). This situation would occur when the affected species is dominant (or co-dominant) in the forest and functionally different from coexistent non-affected species susceptible to increase in abundance and eventually substitute the declining species. For example, in Californian forests affected by sudden oak death, Cobb *et al.* (2013) found that the strongest impact of this disease on the N cycle was not directly caused by the death of the affected *Notholithocarpus densiflorus*, but indirectly due to its substitution by *Umellularia californica*, a species with a higher litterfall %N. In order to predict the long-term effects of forest dieback on biogeochemical cycles, it is essential to compare the 'footprint' of affected and not affected (potentially replacement) species on ecosystem processes (Adams *et al.*, 2010).

The aim of this study was to assess the impact of *Quercus suber* dieback on soil respiration rates and N and P availability in Mediterranean forests of southern Spain. Specifically, we measured *in situ* soil respiration, and ammonium, nitrate and phosphate

availability during three consecutive years in six mixed oak forests affected by the decline of its dominant species, *Q. suber*. In the last decades, a severe decline of evergreen *Quercus* species (*Q. suber* and *Quercus ilex*) in southern Europe has been related to the attack by the exotic pathogen *Phytophthora cinnamomi,* very likely in combination with other stress sources such as climate change-type drought (Brasier, 1996; Camilo-Alves *et al.*, 2013; Corcobado *et al.*, 2013). We explored the direct effects of dieback, comparing the impact on soil variables of *Q. suber* trees with different health status, as well as its potential long-term indirect effects, comparing the "footprint" of *Q. suber* and coexistent late-successional species not affected by dieback (i.e. *Quercus canariensis* and *Olea europaea* var. *sylvestris*). The study was conducted in two contrasted seasons (i.e. spring and summer), given the large intra-annual variation in soil water content typical of Mediterranean ecosystems and its role on biogeochemical cycles (Sardans & Peñuelas, 2005; Asensio *et al.*, 2007). We developed spatially-explicit neighbourhood models that predicted the observed variation in ecosystem processes as a function of the size, identity, health status, abundance and distribution of adult trees in the immediate neighbourhood. This is a novel approach for the study of ecosystem impacts of forest dieback, which is usually conducted at the stand level (e.g. Kizlinski *et al.*, 2002; Orwig *et al.*, 2008; Lovett *et al.*, 2010). The neighbourhood models allowed us to characterize and compare the per capita effects of individual trees with different health status and of different species growing closely intermingled in the canopy. We tested the following three hypotheses. First, we predicted that *Q. suber* dieback would cause reductions in soil respiration rates and P availability given previous works showing reductions in belowground C allocation following disturbance (e.g. Edburg *et al.*, 2011; Nave *et al.*, 2011; Hicke *et al.*, 2012). However, we expected an increase in N availability after dieback due to a reduction in N uptake, as suggested by published studies on other disease and insect-caused tree mortality (e.g. Hobara *et al.*, 2001; Morehouse *et al.*, 2008; Edburg *et al.*, 2011). Second, we predicted different 'footprints' of coexistent species on the studied soil variables given their differences in relevant functional traits such as leaf litter chemistry (e.g. Aponte *et al.*, 2010; Aponte *et al.*, 2011), which would lead to long-term impacts on ecosystems. Third, we predicted that the effects of *Q. suber* dieback on soil processes would be of larger magnitude in spring than in summer, as spring represents the season of higher plant activity in Mediterranean systems (Bonilla & Rodà, 1992; Tang & Baldocchi, 2005). With this work we aim to provide valuable insights that contribute to fill the existent gap in knowledge on the ecosystem-level impacts of tree dieback in Mediterranean forests.

Material and methods

Study site

This study was conducted in Los Alcornocales Natural Park (Southern Spain), considered one of the largest *Quercus suber* L. forests within the Mediterranean Basin (Urbieta *et al.*, 2008). The climate is sub-humid Mediterranean, with mild and humid winters and warm and dry summers. Annual mean temperature ranges from 15.4 to 17.3 ºC and annual mean rainfall varies from 720 to 1100 mm (period 1951-1999, Ninyerola *et al.*, 2005). The hydrological years of the study (i.e. 2010-2012) had contrasting rainfall conditions: the year 2010 was a wet year, with higher than average annual rainfall (1917 mm; 381 mm in spring and 43 mm in summer), the year 2011 was an average year (1145 mm; 363 mm in spring and 9 mm in summer), and 2012 was an extremely dry year (559 mm; 127 mm in spring and 0 mm in summer - data from a meteorological station located at the study area: 5°27′20′′W, 36°26′00′′N). These forests occur on acidic, nutrient-poor, sandy soils, derived from Oligo-Miocene sandstone, but sometimes they are interspersed with clayish soils derived from layers of marl sediments. The flora in the Alcornocales Natural Park is dominated by mixed sclerophyll forests, with *Q. suber* as the main species. In drier lowlands of the park with clayish soils, *Q. suber* coexists with the evergreen *Olea europaea* var. *sylvestris* Brot. forming open woodlands. In moister habitats with sandy soils, *Q. suber* appears intermingled with the deciduous *Quercus canariensis* Willd. forming closed forests. Structurally, open woodlands show lower tree densities and higher solar radiation in the understory than closed forests (Ojeda *et al.*, 2000).

Quercus suber forests within the Natural Park suffer from severe problems of dieback driven by the soil-borne oomycete pathogen *Phytophthora cinnamomi* Rands, one of the most aggressive invasive pathogens on earth (Brasier, 1992; Gómez-Aparicio *et al.*, 2012). This pathogen destroys the root systems, killing fine roots and occasionally producing necrotic bark lesions in large roots (Weste & Marks, 1987). The effect of *P. cinnamomi* in root systems can interact with drought stress and lead to a severe decrease in tree survival (Brasier, 1996; Camilo-Alves *et al.*, 2013; Corcobado *et al.*, 2013).Trees may die suddenly but death often takes several years (Weste & Marks, 1987). No other tree or shrub species in the study area showed evident symptoms of dieback.

Sampling design and neighbourhood characterization

We selected 6 sites within the Natural Park, three situated in open woodlands of *Q. suber* and *O. europaea* and the other three in closed forests dominated by *Q. suber* and *Q. canariensis*. This selection allowed us to explore the ecosystem implications of *Q. suber* dieback in the two main mixed forest types of the region, covering an ample gradient of climate and soil conditions. At each site, a permanent 70 x 70 m plot was established and subdivided into a grid with 49 10 x 10 m subplots. At the centre of each subplot we established a sampling point, with 147 points per forest type (a total of 294).

To characterize local neighbourhoods, we mapped and identified all live and dead trees and shrubs around each sampling point. We determined a neighbourhood as a 15-m radius circle around each sampling point (Gómez-Aparicio *et al.*, 2012; Ibáñez *et al.*, 2015). In each neighbourhood we identified and mapped all trees taller than 1.5 m and with a diameter at breast height (d.b.h.) > 2 cm using a total station Leica TC 407 (Leica Geosystems, Heerbrugg, Switzerland). We measured the d.b.h. of each of the trees mapped (n = 1341 trees). In addition, we classified *Q. suber* trees into three different health categories: healthy, defoliated and dead (Gómez-Aparicio *et al.*, 2012; Ibáñez *et al.*, 2015). Although information on the time of death for *Q. suber* trees was not available, the decline of the species at the study plots is a relatively recent and progressive process, so dead trees (most of them still standing) can be considered to have occurred in the short term (< 10 years).

Biogeochemical measurements

Soil respiration rates were estimated using an alkali trap absorption method (Singh & Gupta, 1977). We chose this static method because it allows extensive replication, and thus could be applied simultaneously in all the 294 sampling points of the study (Singh & Gupta, 1977; Pumpanen *et al.*, 2010). Absorption methods have been effectively used in previous studies and can be reliable for field measurements when they are implemented with due care minimizing possible errors (e.g. Joshi *et al.*, 1991; Gallardo & Schlesinger, 1994; Keith & Wong, 2006; Barba *et al.*, 2013). During spring (May) and summer (August) of 2011 and 2012 we located an alkali trap of 45 ml of NaOH 1.5N placed in a plastic vial of 60 ml (height 66 mm, top diameter 44.2 mm) at each sampling point. Each trap was covered with a cylindrical metal tin (height 153.5 mm, diameter 155 mm) that

was turned upside down and pushed into the soil to a depth of 2 cm (Froment, 1972). After 6 days, traps were closed with a cap and transported to the lab, where the carbon dioxide production was estimated by a titration of the excess of NaOH remaining in the trap with HCl, previous precipitation of carbonates with $BaCl_2$ (Froment, 1972). Soil respiration rates were expressed in gCm^2 $year^{-1}$.

Nutrient availability was also measured at each sampling point using ionic exchange membranes (IEMs, types I-100 and I-200, Electropure Excellion, Laguna Hills, California). This method has been demonstrated to provide a useful alternative to traditional soil extraction for estimating soil inorganic N and P availability (Qian & Schoenau, 2002; Durán *et al.*, 2013). Moreover, nutrient concentrations measured by this method are strongly correlated to plant uptake of those nutrients (Schoenau & Huang, 1991; Ziadi *et al.*, 1999). We estimated soil N and P availability (NH_4−N, NO_3−N and PO_4−P) following the method developed by Durán *et al.* (2008) and Rodríguez *et al.* (2009). Both, anion and cation exchange membranes (2.5 x 2.5 cm) were positioned at a depth of 10 cm with a metal spatula and we ensured good contact between the membranes and the soil. The membranes were incubated for 30 days at each sampling point in spring (May) and summer (August) during 2010, 2011 and 2012. In the lab, the adsorbed nutrients in the membranes were extracted using 25 ml of 2 M KCl in 125ml flasks by orbital spinning for 1h at 200 rpm. We estimated nutrient concentration of the extracts by colorimetry, using a microplate reader (Asys Jupiter−Asys Hitech, Austria). Inorganic N concentration (NH_4−N and NO_3−N) was assessed with the indophenols blue method (Sims *et al.*, 1995) and phosphate concentration (PO_4−P) by the molybdenum blue method (Allen *et al.*, 1986). N and P availability were expressed as μgN(or P) $dm^{-2}day^{-1}$.

Data analysis

Neighbourhood models of soil respiration and nutrient availability - For the analysis of our data we applied likelihood methods and model selection (Johnson & Omland, 2004; Canham & Uriarte, 2006; Gómez-Aparicio & Canham, 2008). We fit separate models for each combination of soil variable (soil respiration rates and ammonium, nitrate and phosphate availability), forest type (woodland and closed forest) and season (spring and summer). Our full model estimated two terms: 1) the average soil variable at each combination of study site and year in the absence of specific effects of neighbouring trees

($a_{\text{Site-Year}}$ in equations 1 and 2), and 2) tree neighbourhood effects on soil variables, which are assumed to vary as a function of a neighbourhood index (NI). We included these neighbourhood effects using both a linear (1) and an exponential (2) model framework (for a similar approach, see Baribault & Kobe, 2011; Gómez-Aparicio *et al.*, 2012):

$$\text{Soil variable} = a_{\text{Site-Year}} + b_{\text{Year}} \times \text{NI} \quad \text{(eqn 1)}$$

$$\text{Soil variable} = a_{\text{Site-Year}} \times \exp(b_{\text{Year}} \times \text{NI}) \quad \text{(eqn 2)}$$

The parameter b_{Year} defines the steepness of the variation in the soil variables due to an increment in NI and was allowed to vary among years to account for inter-annual differences in tree effects. The NI quantifies the net effect of *j=1,…,n* neighbouring trees of *i=1,…,s* species on the soil variable and it is related directly to the size (d.b.h.) of the neighbouring trees and inversely to the distance to neighbours (Gómez-Aparicio & Canham, 2008; Boyden *et al.*, 2012):

$$NI = \sum_{i=0}^{s} \sum_{j=0}^{n} \lambda_i \frac{dbh_{ij}^{\alpha}}{dist_{ij}^{\beta}} \quad \text{(eqn 3)}$$

where dbh_{ij} is the diameter at breast height of each of the *j*th neighbouring tree of the *i*th species and $dist_{ij}$ is the distance of the *j*th neighbouring tree of the *i*th species to the sampling point. The parameters α and β estimated by the model determine the shape of the effect of the tree size (α) and the distance to the neighbour (β) on the index.

In order to explore differences between neighbours of different species or health status on their effects on soil variables, we multiplied the net effect of each individual tree by a per capita coefficient (λ) that ranged from -1 to 1. We tested three different candidate models of decreasing complexity (i.e. decreasing number of λ parameters) to assess the effects of the tree community on the studied soil variables. First, we fit a model that explicitly considered the effect of trees of different health status and species on soil properties ('Health + Tree species' model). This model calculated 4 different λvalues (healthy, defoliated and dead *Q. suber* in both forest types, and *O. europaea* in woodlands or *Q. canariensis* in closed forests). Second, we fit a model that ignored the health status of *Q. suber* and only considered differences among tree species on their impacts on soil variables ('Tree species' model). This model calculated 2 λvalues, one for *Q. suber* and one for the coexisting tree species (*O. europaea* or *Q. canariensis* depending on the forest type). And third, we fit a null model that ignored tree effects on soils ('No tree' model) and therefore calculated soil variables just as a function of the site and year of study.

Moreover, we compared models that considered variability among sites and/or years in 1) background values of soil variables ($a_{Site\text{-}year}$ parameter) and 2) tree effects (b_{Year} parameter) with simpler models that ignored these sources of spatio-temporal variability in soil variables.

Parameter estimation and model selection - Soil variables were modelled with maximum likelihood using a simulated annealing algorithm (Goffe *et al.*, 1994). Model comparisons were conducted following information theory principles and Akaike Information Criterion corrected for small sample size (AICc, Burnham & Anderson, 2002). Lower AIC_c values indicate best model performance. Asymptotic two-unit support intervals were calculated for each estimated parameter. The goodness-of-fit of the best models was determined with the R^2 of the regression between observed and predicted data. The slope of that regression was used to measure model bias, with an unbiased prediction having a slope ~1. Spatial autocorrelation in the residuals was analyzed with Mantel tests (Legendre & Fortin, 1989), whereas temporal correlation was analyzed exploring lack of patterns in the plots of residuals against year. However, it is important to highlight that likelihood-based inference is very robust even in cases of lack of independence, with deviations from independence mostly resulting in underestimates of variance terms but not in biased parameters values (Hubbell *et al.*, 2001; Uriarte *et al.*, 2004). All statistical analysis were performed using R 3.0.2 (R Core Team, 2013) and the 'likelihood' package (Murphy, 2012).

Results

Soil respiration rates

All models produced unbiased estimates of soil respiration rates (i.e. slopes of observed vs. predicted were close to 1) and explained from 25 to 68% of the data variation. Models did not show spatial or temporal autocorrelation in their residuals with one exception (the null model in closed forests in spring, see Table S1 and Fig. S1 in Supporting Information). Most of the best models (3 of 4) included the effects of the tree community (i.e. 'Health + Tree species' or 'Tree species' models) on respiration rates. Differential effects of *Q. suber* trees depending on their health status were detected in the two forest types, but only in summer (i.e. the 'Health + Tree species' model had the lowest AIC_c,

Table 1). Neighbourhoods dominated by healthy *Q. suber* trees had higher soil respiration rates than those dominated by defoliated or dead *Q. suber* trees (Figs. 1b,d). We detected similar soil respiration rates in neighbourhoods dominated by healthy *Q. suber* compared to neighbourhoods dominated by the coexistent species (see overlapping values of λ parameters in Tables S2 and S3 in Supporting Information) (Fig. 1). No inter-annual differences were found in the tree effects (no differences in parameter b_{Year} among years, Tables S2 and S3).

Table 1 Comparison of alternative models for soil respiration rates and N and P availability in the two forest types (woodlands and closed forests) and seasons (SPR, spring; SUM, summer) using the Akaike Information Criterion corrected for small sample sizes (AIC_c). □AIC_c represents the differences between the AIC_c of the model and the AIC_c of the best model (i.e. the model with the lowest AICc, indicated in bold). 'Health + Tree species' models include differences among tree species and health status in their effects on soils, 'Tree species' models consider only differences among tree species, and 'No tree' models do not consider any tree effects. b_{Year} (eqn. 1 and 2 in the text) shows the existence of inter-annual differences in tree effects on soil variables (Y, YES; N, NO). 'Mod' indicates a linear (Lin) or an exponential (Exp) relationship between soil variables and tree effects. 'Par' is the total number of parameters in the best model. The slope ('SL') and R^2 for the relationship between predicted and observed values are also given.

Type of forest	Soil variable	Season	ΔAICc			b_{Year}	Mod	Par	SL	R^2
			Health + Tree species	Tree species	No tree					
Woodlands	CO_2	SPR	4.89	**0.00**	3.48	N	Exp	12	1.00	0.25
		SUM	**0.00**	2.24	12.64	N	Lin	14	0.97	0.68
	NH_4^+	SPR	7.59	**0.00**	2.88	N	Lin	12	0.96	0.43
		SUM	**0.00**	41.91	113.20	Y	Lin	19	1.06	0.36
	NO_3^-	SPR	6.27	**0.00**	8.33	N	Lin	12	0.96	0.08
		SUM	**0.00**	13.34	22.00	N	Lin	11	0.99	0.18
	PO_4^{3-}	SPR	7.58	**0.00**	1.91	N	Exp	15	1.00	0.31
		SUM	**0.00**	5.91	19.67	N	Exp	17	0.94	0.17
Closed forests	CO_2	SPR	13.25	8.94	**0.00**	-	-	7	0.99	0.42
		SUM	**0.00**	50.95	61.20	N	Lin	14	0.97	0.67
	NH_4^+	SPR	**0.00**	17.99	22.20	N	Lin	17	0.92	0.18
		SUM	**0.00**	12.63	13.71	Y	Lin	19	0.97	0.29
	NO_3^-	SPR	**0.00**	65.70	105.32	N	Lin	14	1.09	0.30
		SUM	**0.00**	26.45	58.46	N	Lin	17	0.99	0.13
	PO_4^{3-}	SPR	8.00	5.71	**0.00**	-	-	10	1.00	0.18
		SUM	**0.00**	66.53	136.15	Y	Lin	19	0.97	0.17

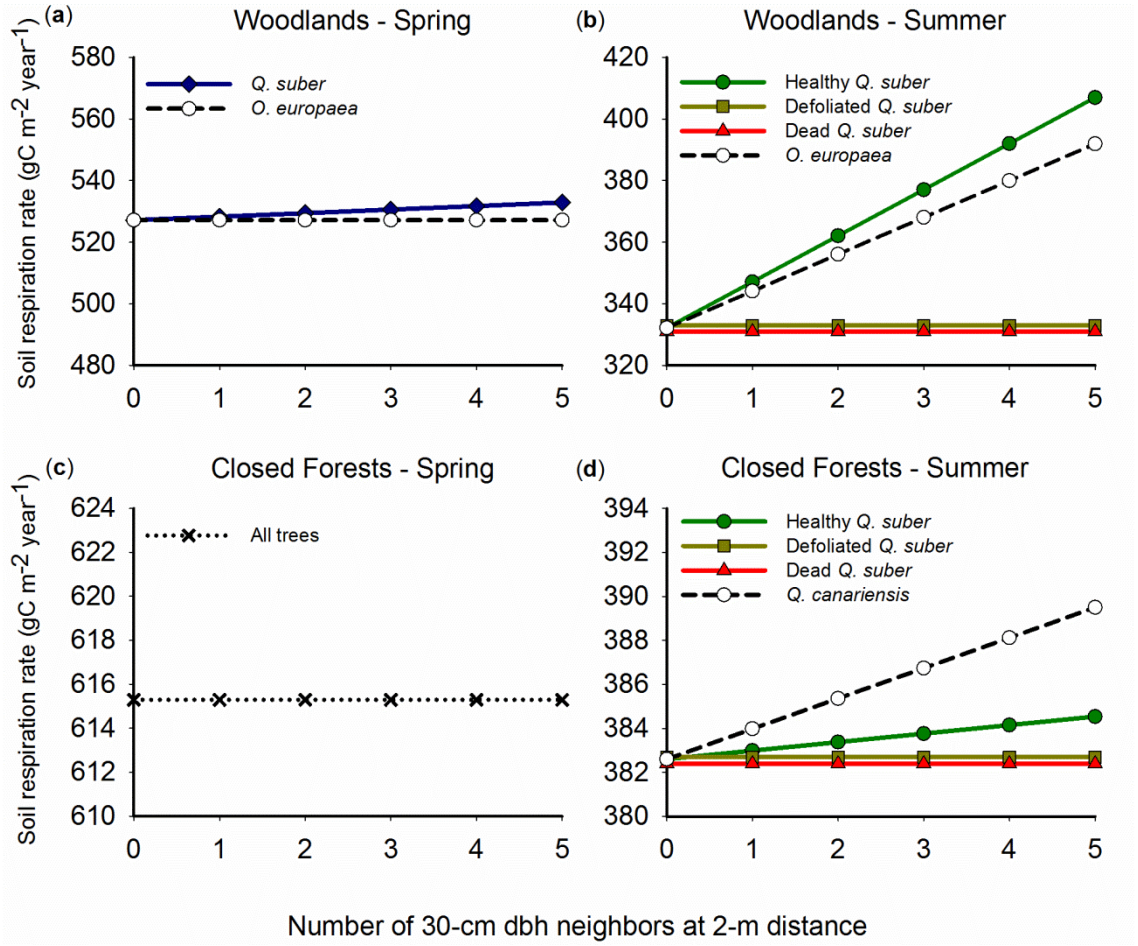


Figure 1 Predicted effects of variation in neighbour identity and quantity on soil respiration rates in woodlands during spring (a) and summer (b), and in closed forests during spring (c) and summer (d). Predictions were done using parameter values from best models (Supporting Information Tables S2, S3). The number (0-5) of average-sized neighbours (i.e. d.b.h. = 30 cm) at 2-m distance represent the range of neighbour abundance found in our forests for that distance.

We found important variation among seasons and forest types in the characteristics of the footprint of individual trees on soil respiration rates. In woodland sites, a large α value (>2) in spring indicated neighbour effects that were disproportionally large relative to the tree size, whereas a α~0 in summer indicated effects that scaled with neighbour density, regardless of the size of individual trees (Table S3). The decay in the tree effect in woodlands (controlled by parameter β) was steeper in spring than in summer (Fig. 2a, Table S3). In closed forests (where tree effects were only detected in summer), the footprint of individual trees on soil properties increased strongly with tree size and decreased steeply with distance (Fig. 2a, Table S3).

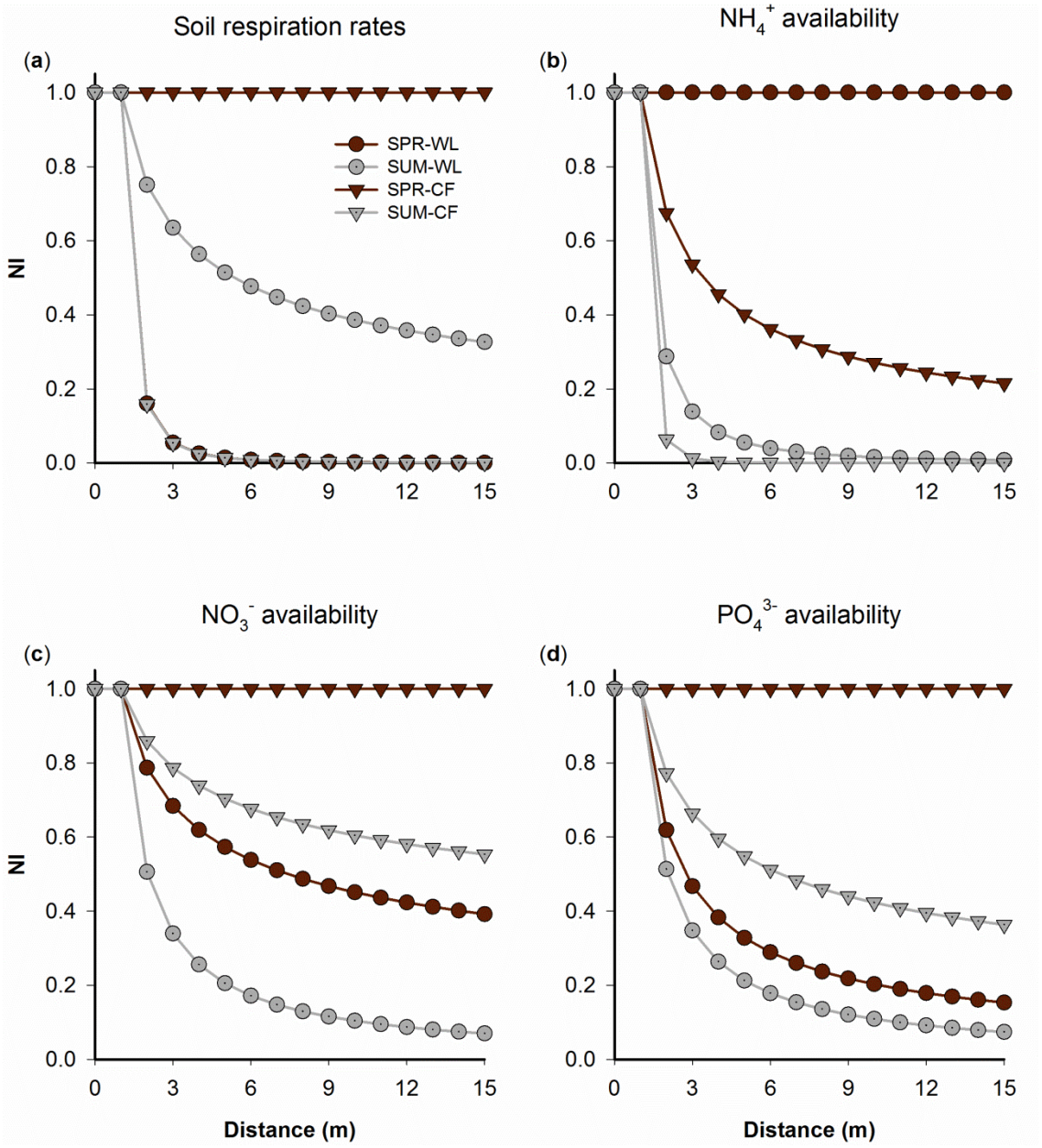


Figure 2 Predicted decline in the neighbourhood index (NI) as a function of distance to a neighbour for the soil respiration rates (a) and NH_4^+ (b), NO_3^- (c) and PO_4^{3-} availability (d). NI is calculated using equation 3 and values of β parameter given in Supporting Information Table S2 and S3 for best models ($\alpha = 0$ and $\lambda = 1$ for simplicity of presentation of results). Season: SPR = spring and SUM = summer. Forest type: WL = woodlands and CF = closed forests.

Soil N availability

Models produced unbiased estimates of NH_4^+ and NO_3^- availability (slope ~ 1) and accounted from 8 to 43% of the variation in the data. Model residuals did not show temporal or spatial autocorrelation (Table S1, Fig. S1). For all combinations of nitrogen variable, season and forest type, models that considered the effects of the tree community offered a much better fit to the data than models that ignored these effects (Table 1). Most of those models (6 of 8) also included differential impacts of *Q. suber* trees varying in health status (i.e. the 'Health + Tree species' model had the lowest AICc). In woodlands, NH_4^+ and NO_3^- availability was lower in neighbourhoods dominated by dead and defoliated trees than in neighbourhoods dominated by healthy *Q. suber* trees (Figs. 3b and 4b). However, the opposite pattern was detected in closed forests: NH_4^+ and NO_3^- availability was higher in neighbourhoods dominated by dead trees than in those dominated by healthy trees (Figs. 3c,d and 4c,d). Differences in the effects of neighbours with different health status were detected in both seasons, but more consistently in summer. Among species, our results showed differential impacts of healthy *Q. suber* and the coexistent tree species on N variables, as indicated by non-overlapping λ values in most situations (Tables S2 and S3). In woodlands, NH_4^+ and NO_3^- availability was higher in *Q. suber* than in *O. europea* neighbourhoods in most cases (Figs. 3a,b and 4a,b). In closed forests, on the contrary, *Q. suber* neighbourhoods had lower NH_4^+ and NO_3^- availability than neighbourhoods dominated by the co-existent *Q. canariensis*, but these differences disappeared in summer (Figs. 3c,d and 4c,d). In general, no differences among years were detected in tree effects.

The influence of neighbour size (α parameter in the NI function (equation 3)) on N availability was quite variable among forms of nitrogen, seasons and forest types. The effect of neighbour size on N availability varied from disproportionally large effects relative to tree size (e.g. NO_3^- in summer in woodlands) to virtually null (e.g. NO_3^- in spring in woodlands) for which N availability was only proportional to tree density (Tables S2 and S3). The values of the β parameter (which controls the decline in neighbour effects with distance from the tree) were always higher in spring than in summer, indicating much more localized tree effects on N availability in spring. Among forest types, the decay of tree effects with distance was steeper in woodlands than in closed forests for NO_3^-, but not for NH_4^+ (Figs. 2b,c).

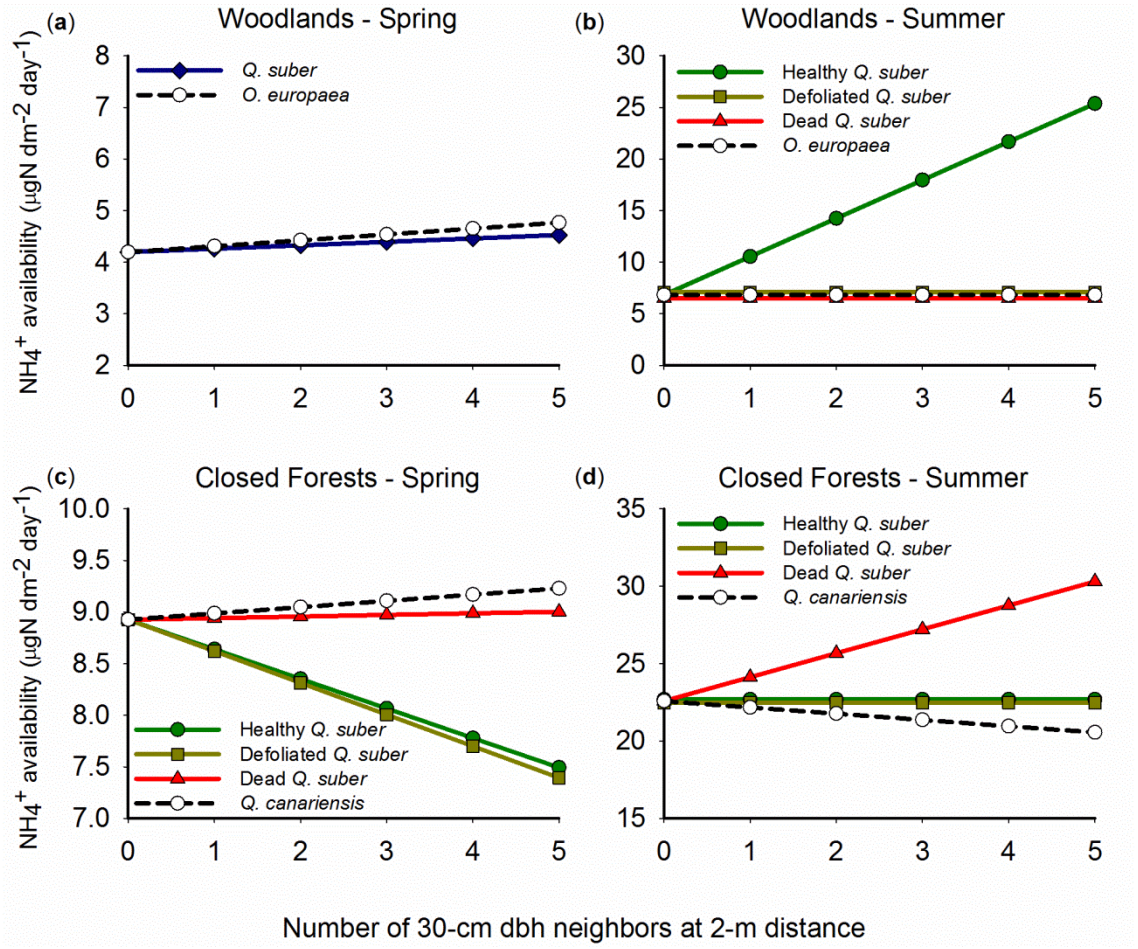


Figure 3 Predicted effects of variation in neighbour identity and quantity on NH_4^+ availability in woodlands during spring (a) and summer (b), and in closed forests during spring (c) and summer (d). Predictions were done using parameter values from best models (Supporting Information Tables S2, S3). The number (0-5) of average-sized neighbours (i.e. d.b.h. = 30 cm) at 2-m distance represent the range of neighbour abundance found in our forests for that distance.

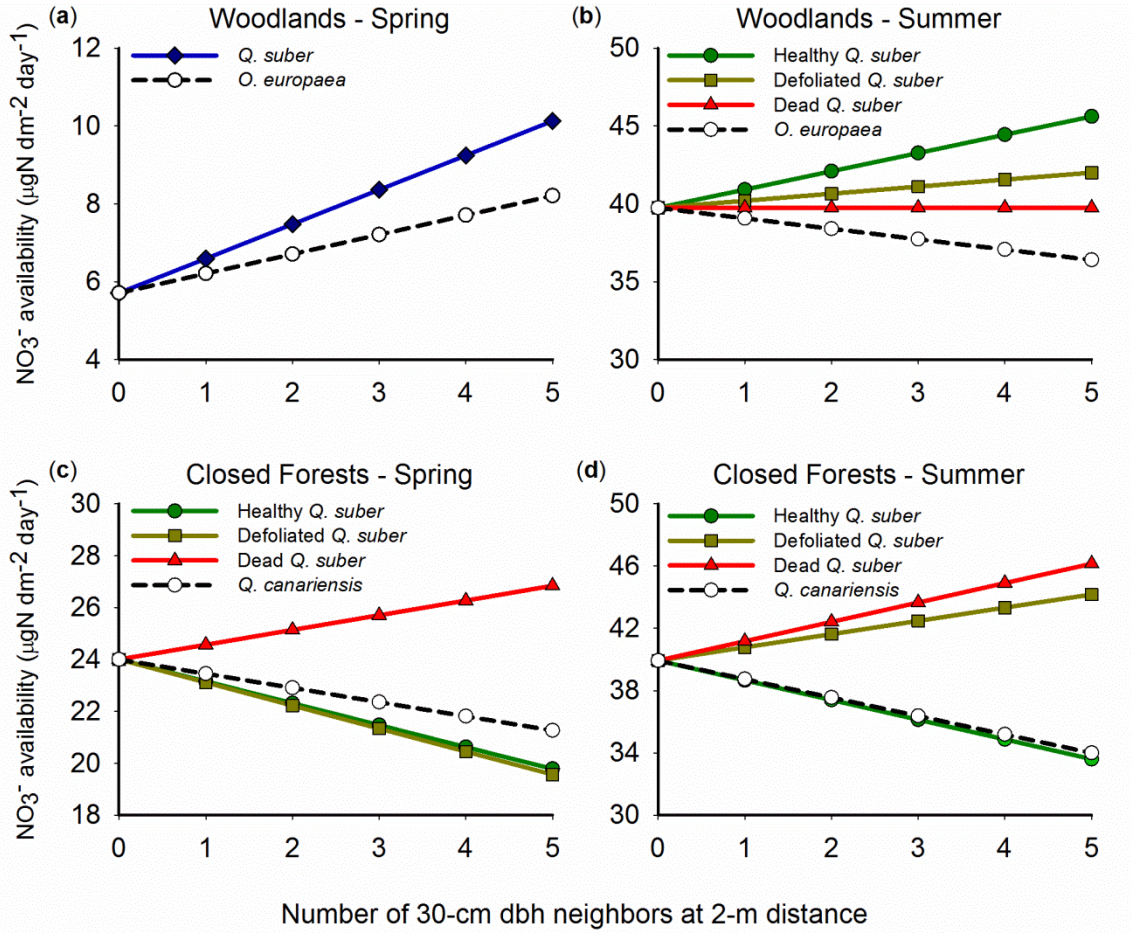


Figure 4 Predicted effects of variation in neighbour identity and quantity on NO_3^-availability in woodlands during spring (a) and summer (b), and in closed forests during spring (c) and summer (d). Predictions were done using parameter values from best models (Supporting Information Tables S2, S3). The number (0-5) of average-sized neighbours (i.e. d.b.h. = 30 cm) at 2-m distance represent the range of neighbour abundance found in our forests for that distance.

Soil P availability

All models produced unbiased estimates of PO_4^{3-} availability (slope ~ 1) and explained from 17 to 31% of its variation (Table 1). Model residuals did not show temporal or spatial autocorrelation, except for a slight spatial autocorrelation for closed forests in summer (Table S1, Fig. S1). Most of the best models (3 of 4) included the effects of the tree community on PO_4^{3-} availability. However, differences among *Q. suber* trees of different health status were only found in summer (i.e. lowest AICc for the 'Health + Tree species' model, Table 1). In both forest types, neighbourhoods dominated by defoliated and dead trees had lower PO_4^{3-} availability than neighbourhoods dominated by healthy *Q. suber* trees (Figs. 5b,d). Among species, healthy *Q. suber* neighbourhoods had generally higher levels of PO_4^{3-} availability than *O. europaea* (woodlands) and *Q. canariensis* (closed

forests) neighbourhoods, but this effect varied between seasons (Fig. 5, see λ in Tables S2 and S3). No differences in the tree effects were detected among years, except for woodlands in summer (see b_{Year} in Tables S2 and S3).

The influence of neighbour size on the effect of trees on PO_4^{3-} availability varied among seasons and forest types. In woodlands, the effect of neighbour size on the influence of trees was larger in spring (α~1.5) than in summer (α~0.5) (Table S2). In closed forests, where the effect of trees was detected just in summer, tree size had a strong effect on PO_4^{3-} availability (Table S3). The decay of tree effect in distance was always steeper in summer than in spring and in woodlands than in closed forests (Fig. 2d, Tables S2 and S3).

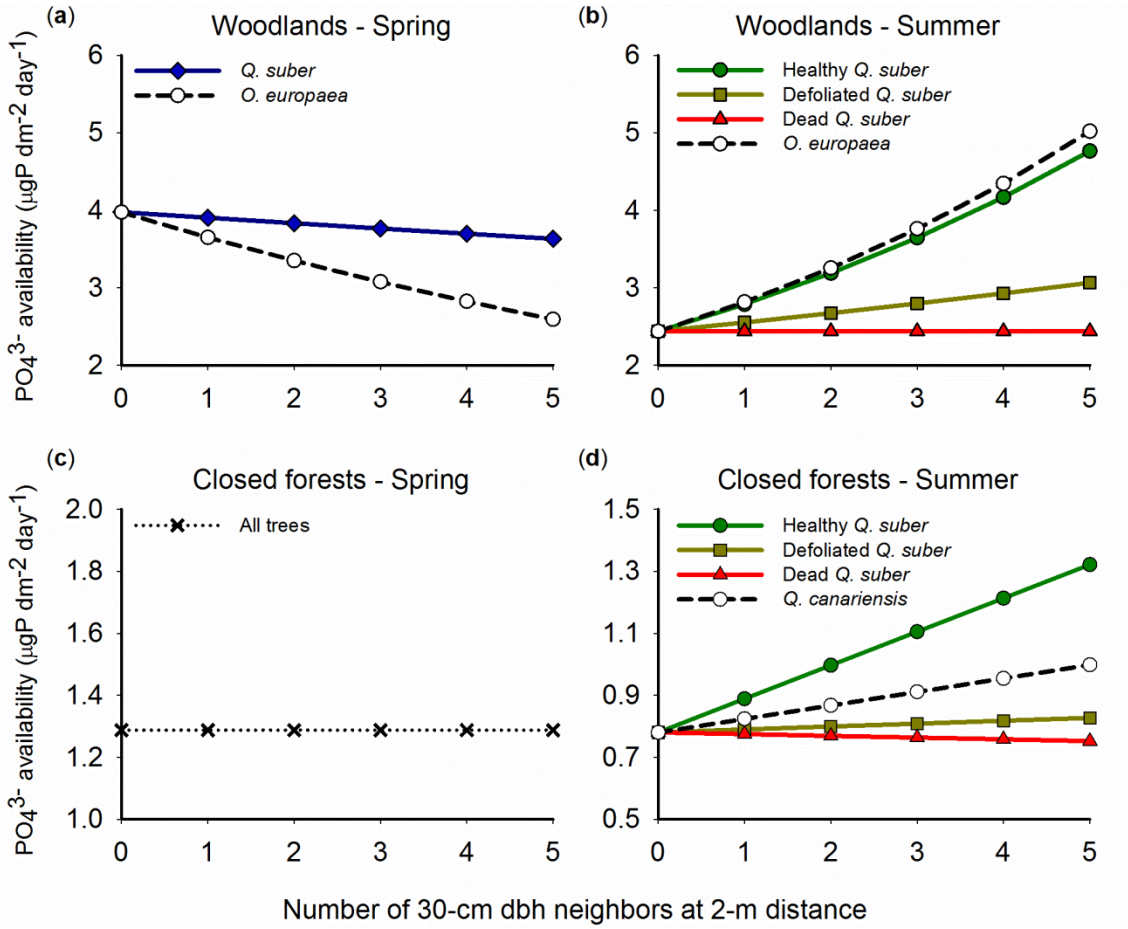


Figure 5 Predicted effects of variation in neighbour identity and quantity on PO_4^{3-}availability in woodlands during spring (a) and summer (b), and in closed forests during spring (c) and summer (d). Predictions were done using parameter values from best models (Supporting Information Tables S2, S3). The number (0-5) of average-sized neighbours (i.e. d.b.h. = 30 cm) at 2-m distance represent the range of neighbour abundance found in our forests for that distance.

Discussion

Our study provides compelling evidence that *Q. suber* dieback leads to important alterations in biogeochemical cycles of Mediterranean oak forests. We found a strong spatial concordance among the distribution and health status of individual trees and the soil variables studied (i.e. soil respiration rates and nutrient availability) consistently across space (i.e. different forest types) and time (i.e. different years and seasons). To our knowledge this study represents the first attempt to connect complex neighbourhoods (i.e. composed by trees of different species, sizes and health status) with the spatiotemporal variability of soil processes, allowing the estimation of per-capita tree effects on ecosystems in a context of tree dieback.

Direct effects of Q. suber dieback on biogeochemical cycles

Our results indicate that the decline and death of *Q. suber* translate into an important reduction in soil respiration rates, in agreement with our first hypothesis. In fact, our models predicted reductions in soil respiration rates as high as 20% in neighbourhoods dominated by dead trees compared to neighbourhoods dominated by healthy trees (Fig. 1). These findings might be linked to a decrease in root and rhizosphere respiration in the first years following disturbance (Nuckolls *et al.*, 2009; Edburg *et al.*, 2011; Flower *et al.*, 2013). Root pathogens as *P. cinnamomi* destroy the root system and reduces tree productivity (Cherubini *et al.*, 2002). This attack could lead to lower belowground C allocation and autotrophic respiration, as it has been demonstrated in girdling experiments simulating pathogen attacks (Högberg & Read, 2006; Nuckolls *et al.*, 2009; Levy-Varon *et al.*, 2012; Levy-Varon *et al.*, 2014). Recent literature on insect-driven tree mortality has suggested that forest dieback might result in large alterations in forest-atmosphere C exchange, shifting the role of forests from a C sink to a C source (Kurz *et al.*, 2008; Hicke *et al.*, 2012). However, our results suggest that in forests affected by pathogen-driven mortality a decrease in total soil respiration rates might attenuate C losses, at least in the short term, weakening the impact of pathogen-induced tree mortality on the net C exchange between the forest and the atmosphere.

Quercus suber dieback altered both N and P availability, but the impacts on P were of larger magnitude than on N. Our models predicted reductions in P as high as 50% in neighbourhoods dominated by dead *Q. suber* trees compared to healthy neighbourhoods (Fig. 4). Therefore, these results confirm our first hypothesis and are consistent with a

mechanistic explanation based on a reduction in root exudation and rhizodeposition after mortality (Högberg *et al.*, 2001; Xiong *et al.*, 2011). The decrease in P availability as a consequence of a reduction in root activity after *Q. suber* dieback would support the idea that root phosphatase plays a key role in P availability for plants and microorganisms in Mediterranean forest systems (Schneider *et al.*, 2001). Our results also agree with those obtained by the only previous study that has analysed the implications of *P. cinnamomi*-induced plant mortality on P availability (Shearer *et al.*, 2009). The reductions in P caused by dieback are of particular relevance in Mediterranean systems, where P is usually considered the main limiting nutrient (e.g. Sardans *et al.*, 2004; Morillas *et al.*, 2012) and has been shown to constrain microbial biomass, tree growth and seedling performance (Sheriff *et al.*, 1986; Gallardo & Schlesinger, 1994; Gómez-Aparicio *et al.*, 2008). Therefore, a decrease in P availability as a consequence of tree dieback would limit even further demographic and ecosystem processes in Mediterranean forests.

Unlike P, the sign of the effect of *Q. suber* dieback on N availability varied among forest types (i.e. woodlands vs. closed forests). Our first hypothesis of positive effects of tree mortality on N availability was supported only in closed forests. This hypothesis was based on previous studies that showed a reduction in N uptake following insect outbreaks or drought-induced mortality (e.g. Kizlinski *et al.*, 2002; Clow *et al.*, 2011; Wang *et al.*, 2012). The fact that our hypothesis was not supported in open woodlands suggests that mechanisms other than plant uptake were driving N availability in these systems. In particular, post-mortality alterations of microclimatic conditions could explain the results found in woodlands. In these forests, gaps opened after tree death were of higher extent than in closed forests due to their lower tree density, leading to much stronger changes in microclimate (i.e. higher light and lower moisture availability, J.M. Ávila *et al.* unpublished data). These microclimatic alterations in large gaps could bring a reduction in N mineralization rates and N availability (Zhang & Zak, 1995). Overall, an important implication of our results is that the sign of the effect of tree dieback on essential nutrients such as N and P can be opposite, depending very much on forest structure. This decoupling between the N and P cycles might in turn impact the C cycle, with important negative effects on primary productivity and organic matter decomposition (Finzi *et al.*, 2011).

Our neighbourhood approach allowed us to assess the effect of tree size and distance to neighbour trees on the studied soil variables. Previous studies have highlighted the strong influence of tree size in explaining the spatial patters of soil respiration or nutrient

availability (Ludwig *et al.*, 2004; Søe & Buchmann, 2005). Although we found a high variability in the effects of neighbour size on soil respiration rates and nutrient availability, our results make a case for the disproportionately large effect of big trees on the studied variables ($\alpha > 2$). This finding, together with the fact that *Phytophthora*-driven mortality usually affects medium to large size canopy trees (Cobb *et al.*, 2012), implies that *Q. suber* dieback is a disturbance with a large capacity to alter ecosystem function. Regarding the role of distance, we found a strong variability in the shape of the tree footprints on soil variables among forest types and seasons (Fig. 2). Tree effects on the studied soil variables extended generally further in closed forests than in woodlands and in spring than in summer. The strong spatial (forest type) and temporal (seasonal) variability in the spatial extension of tree effects confirms for Mediterranean forests the inherent complexity of the spatial component of tree effects on ecosystems (Gómez-Aparicio & Canham, 2008), and suggests that it should be considered in future studies in order to gain a solid understanding of dieback impacts on ecosystem functioning.

Indirect effects of Q. suber dieback on biogeochemical cycles

In the long term, the process of secondary succession after tree mortality might induce species replacements that could lead to indirect impacts on ecosystem processes (e.g. Lovett *et al.*, 2006; Loo, 2009; Lovett *et al.*, 2010). In agreement with our second hypothesis, we found differences among species in their effects on soil variables that could translate into important indirect changes in biogeochemical cycles due to the substitution of *Q. suber* by non-declining coexistent species. However, our models also indicated that the direction of such changes might vary strongly among ecosystem processes. Thus, we did not detect species-specific differences in soil respiration rates, likely influenced by similar root maintenance respiration rates among species (Martínez *et al.*, 2002), but found important differences in nutrient availability (i.e. N and P). Such differences might be related to contrasting litter quality between species. Neighbourhoods dominated by *O. europaea* - a species characterized by its low litter quality (Rodríguez Pleguezuelo *et al.*, 2009, J.M. Ávila *et al.*, unpublished data) - had soils with lower N and P availability than *Q. suber* neighbourhoods, whereas neighbourhoods dominated by *Q. canariensis* - with a nutrient-rich litter (Aponte *et al.*, 2011) - had soils with higher N availability than *Q. suber* neighbourhoods. A relevant implication of this result is that the indirect effects of the mortality of a given tree species will be strongly dependent on the

identity of the replacement species, leading to different long-term ecosystem implications that can vary from acceleration to deceleration of nutrient cycles.

Temporal variations in the effects of Q. suber dieback on biogeochemical cycles

In Mediterranean ecosystems, the strong inter- and intra-annual variability of soil water availability have a profound effect on ecosystem functioning since water is the main factor controlling above- and belowground processes such as photosynthesis, decomposition or soil respiration (Asensio *et al.*, 2007; Galmés *et al.*, 2007). However, how the temporal variation of water availability affects the impacts of forest dieback on ecosystems has not been explored yet. Our models showed low inter-annual but high intra-annual variation in the ecosystem impacts of tree dieback. We detected no substantial differences in the impacts of tree dieback among the studied years, even though they had contrasting climatic conditions (see Study site). However, the impacts of *Q. suber* dieback varied strongly within the year. In fact, the effects of tree health status on soil variables were detected mainly in summer, contrary to our third hypothesis. The differences in the effects between seasons could be explained by the fact that during spring not only the tree activity is high, but also the activity of microbes and herbaceous vegetation (Jackson *et al.*, 1988). For instance, a previous study conducted in a Mediterranean oak savanna ecosystem found that an increase in grass cover during spring caused a reduction in the spatial variation of soil respiration rates, weakening the differences in soil CO_2 fluxes among soils under and outside oak canopies (Tang & Baldocchi, 2005). Therefore, the likelihood to detect differences in C and nutrient cycling between healthy and declining or dead trees would be much lower during spring than during summer. Tree neighbourhood effects might also be weaker in spring as a consequence of the high soil moisture content in this season, which can keep the soil well connected hydrologically leading to high respiration and mineralization rates in the whole system (Gallart *et al.*, 2002). The strong seasonal variability of the ecosystem consequences of *Q. suber* dieback highlights the importance of considering the temporal component of these processes to obtain a comprehensive understanding of the implications of this disturbance for Mediterranean systems.

Conclusions

Forest dieback is a concerning issue worldwide and particularly in water-limited Mediterranean regions, where several forests are affected by pathogen-induced and/or drought-induced tree mortality (Carnicer *et al.*, 2011; Garbelotto & Pautasso, 2012). This study provides novel evidence on the effects of the spatial distribution of intermingled trees of different species and health status on three of the most important biogeochemical cycles (i.e. C, N and P). Moreover, to our knowledge this is the first study that assesses the impacts of tree dieback on C, N and P cycles simultaneously. Our results support that the decline and mortality of a particular species in a mixed forest might have important short- and long-term impacts on different biogeochemical cycles that do not always go in the same direction (i.e. varying from positive to negative effects), leading to potential decoupling among cycles. Our neighbourhood models also suggested that large trees - which are those more frequently affected by decline and mortality (Griffin *et al.*, 2011; Cobb *et al.*, 2012) - had disproportionate impacts on soil respiration and nutrient availability, conferring to the process of forest dieback an extremely high capacity to modify biogeochemical cycles.

The findings of this study are of special relevance in a context of tree dieback induced by *P. cinnamomi*. This pathogen, considered one of the most aggressive invasive species on earth, has invaded most temperate and subtropical regions in the world (Hansen, 2015) and has already devastated important areas in Australia (Cahill *et al.*, 2008) and Europe (Brasier, 1996). The negative effects of this pathogen are expected to increase in the near future due to the positive effects of warming temperatures on pathogen activity and to the expansion of its potential range to higher latitudes where low temperatures currently limit its establishment (Brasier, 1996; Sturrock *et al.*, 2011). Therefore, the spatial extension and the magnitude of the ecosystem impacts of tree mortality detected in this work might be expected to increase in future years, which could have profound implications for ecosystem functioning in forests worldwide.

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Data accessibility

Data available from the Dryad Digital Repository doi:10.5061/dryad.6nd4k (Avila *et al.* 2016)

References

Adams HD, Macalady AK, Breshears DD, Allen CD, Stephenson NL, Saleska SR, Huxman TE. 2010. Climate-Induced Tree Mortality: Earth System Consequences. Eos, Transactions American Geophysical Union 91(17): 153-154.

Allen CD. 2009. Climate-induced forest dieback: an escalating global phenomenon. Unasylva 231(232): 43-49.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim JH, Allard G, Running SW, Semerci A, Cobb N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management 259(4): 660-684.

Allen SE, Grimshaw HM, Rowland AP 1986. Chemical analysis. In: Moore PD, Chapman SB eds. Methods in Plant Ecology. Oxford: Blackwell Scientific Publications, 285-344.

Anderegg WRL, Kane JM, Anderegg LDL. 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. Nature Climate Change 3(1): 30-36.

Aponte C, García LV, Pérez-Ramos IM, Gutiérrez E, Marañón T. 2011. Oak trees and soil interactions in Mediterranean forests: A positive feedback model. Journal of Vegetation Science 22(5): 856-867.

Aponte C, Marañón T, García LV. 2010. Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth. Biogeochemistry 101(1): 77-92.

Asensio D, Peñuelas J, Ogaya R, Llusià J. 2007. Seasonal soil and leaf CO2 exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. Atmospheric Environment 41(11): 2447-2455.

Attiwill PM, Adams MA. 1993. Nutrient cycling in forests. New Phytologist 124(4): 561-582.

Barba J, Curiel Yuste J, Martínez-Vilalta J, Lloret F. 2013. Drought-induced tree species replacement is reflected in the spatial variability of soil respiration in a mixed Mediterranean forest. Forest Ecology and Management 306: 79-87.

Baribault TW, Kobe RK. 2011. Neighbour interactions strengthen with increased soil resources in a northern hardwood forest. Journal of Ecology 99(6): 1358-1372.

Block CE, Knoepp JD, Fraterrigo JM. 2013. Interactive effects of disturbance and nitrogen availability on phosphorus dynamics of southern Appalachian forests. Biogeochemistry 112(1-3): 329-342.

Bonilla D, Rodà F. 1992. Soil-nitrogen dynamics in a holm oak forest. Vegetatio 100: 247-257.

Boyden S, Montgomery R, Reich PB, Palik B. 2012. Seeing the forest for the heterogeneous trees: stand-scale resource distributions emerge from tree-scale structure. Ecological Applications 22(5): 1578-1588.

Brasier CM. 1992. Oak tree mortality in Iberia. Nature 360(6404): 539.

Brasier CM. 1996. Phytophthora cinnamomi and oak decline in southern Europe. Environmental constraints including climate change. Annals of Forest Science 53(2-3): 347-358.

Brown M, Black TA, Nesic Z, Foord VN, Spittlehouse DL, Fredeen AL, Grant NJ, Burton PJ, Trofymow JA. 2010. Impact of mountain pine beetle on the net ecosystem production of lodgepole pine stands in British Columbia. Agricultural and Forest Meteorology 150(2): 254-264.

Burnham KP, Anderson DR. 2002. Model Selection and Multi-Model Inference : A Practical Information-Theoretic Approach. Secaucus, NJ, USA: Springer.

Cahill DM, Rookes JE, Wilson BA, Gibson L, McDougall KL. 2008. Phytophthora cinnamomi and Australia's biodiversity: impacts, predictions and progress towards control. Australian Journal of Botany 56(4): 279-310.

Camilo-Alves CSP, da Clara MIE, Ribeiro NA. 2013. Decline of Mediterranean oak trees and its association with Phytophthora cinnamomi: a review. European Journal of Forest Research 132(3): 411-432.

Canham CD, Uriarte M. 2006. Analysis of neighborhood dynamics of forest ecosystems using likelihood methods and modeling. Ecological Applications 16(1): 62-73.

Carnicer J, Coll M, Ninyerola M, Pons X, Sánchez G, Peñuelas J. 2011. Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proceedings of the National Academy of Sciences of the United States of America 108(4): 1474-1478.

Clow DW, Rhoades C, Briggs J, Caldwell M, Lewis WM. 2011. Responses of soil and water chemistry to mountain pine beetle induced tree mortality in Grand County, Colorado, USA. Applied Geochemistry 26(SUPPL.): S174-S178.

Cobb RC, Eviner VT, Rizzo DM. 2013. Mortality and community changes drive sudden oak death impacts on litterfall and soil nitrogen cycling. New Phytologist 200(2): 422-431.

Cobb RC, Filipe JAN, Meentemeyer RK, Gilligan CA, Rizzo DM. 2012. Ecosystem transformation by emerging infectious disease: Loss of large tanoak from California forests. Journal of Ecology 100(3): 712-722.

Corcobado T, Cubera E, Moreno G, Solla A. 2013. Quercus ilex forests are influenced by annual variations in water table, soil water deficit and fine root loss caused by Phytophthora cinnamomi. Agricultural and Forest Meteorology 169: 92-99.

Cherubini P, Fontana G, Rigling D, Dobbertin M, Brang P, Innes JL. 2002. Tree-life history prior to death: two fungal root pathogens affect tree-ring growth differently. Journal of Ecology 90(5): 839-850.

Durán J, Delgado-Baquerizo M, Rodríguez A, Covelo F, Gallardo A. 2013. Ionic exchange membranes (IEMs): A good indicator of soil inorganic N production. Soil Biology & Biochemistry 57: 964-968.

Durán J, Rodríguez A, Fernández-Palacios JM, Gallardo A. 2008. Changes in soil N and P availability in a Pinus canariensis fire chronosequence. Forest Ecology and Management 256(3): 384-387.

Edburg SL, Hicke JA, Brooks PD, Pendall EG, Ewers BE, Norton U, Gochis D, Gutmann ED, Meddens AJH. 2012. Cascading impacts of bark beetle-caused tree mortality on coupled biogeophysical and biogeochemical processes. Frontiers in Ecology and the Environment 10(8): 416-424.

Edburg SL, Hicke JA, Lawrence DM, Thornton PE. 2011. Simulating coupled carbon and nitrogen dynamics following mountain pine beetle outbreaks in the western United States. Journal of Geophysical Research G: Biogeosciences 116(4): G04033.

Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppel BD, Knoepp JD, Lovett GM, Mohan J, Orwig DA, Rodenhouse NL, Sobczak WV, Stinson KA, Stone JK, Swan CM, Thompson J, Von Holle B, Webster JR. 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Frontiers in Ecology and the Environment 3(9): 479-486.

Finzi AC, Austin AT, Cleland EE, Frey SD, Houlton BZ, Wallenstein MD. 2011. Responses and feedbacks of coupled biogeochemical cycles to climate change: examples from terrestrial ecosystems. Frontiers in Ecology and the Environment 9(1): 61-67.

Flower CE, Knight KS, Gonzalez-Meler MA. 2013. Impacts of the emerald ash borer (Agrilus planipennis Fairmaire) induced ash (Fraxinus spp.) mortality on forest carbon cycling and successional dynamics in the eastern United States. Biological Invasions 15(4): 931-944.

Froment A. 1972. Soil respiration in a mixed oak forest. Oikos 23(2): 273-277.

Galmés J, Medrano H, Flexas J. 2007. Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. New Phytologist 175(1): 81-93.

Gallardo A, Schlesinger WH. 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biology and Biochemistry 26(10): 1409-1415.

Gallart F, Llorens P, Latron J, Regüés D. 2002. Hydrological processes and their seasonal controls in a small Mediterranean mountain catchment in the Pyrenees. Hydrology and Earth System Sciences 6(3): 527-537.

Garbelotto M, Pautasso M. 2012. Impacts of exotic forest pathogens on Mediterranean ecosystems: four case studies. European Journal of Plant Pathology 133(1): 101-116.

George TS, Fransson A-M, Hammond JP, White PJ 2011. Phosphorus Nutrition: Rhizosphere Processes, Plant Response and Adaptations. In: Bünemann E, Oberson A, Frossard E eds. Phosphorus in Action: Springer Berlin Heidelberg, 245-271.

Goffe WL, Ferrier GD, Rogers J. 1994. Global optimization of statistical functions with simulated annealing. Journal of Econometrics 60(1-2): 65-99.

Gómez-Aparicio L, Canham CD. 2008. Neighborhood models of the effects of invasive tree species on ecosystem processes. Ecological Monographs 78(1): 69-86.

Gómez-Aparicio L, Ibáñez B, Serrano MS, De Vita P, Ávila JM, Pérez-Ramos IM, García LV, Sánchez ME, Marañón T. 2012. Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration. New Phytologist 194(4): 1014-1024.

Gómez-Aparicio L, Pérez-Ramos IM, Mendoza I, Matías L, Quero JL, Castro J, Zamora R, Marañón T. 2008. Oak seedling survival and growth along resource gradients in Mediterranean forests: Implications for regeneration in current and future environmental scenarios. Oikos 117(11): 1683-1699.

Griffin JM, Turner MG, Simard M. 2011. Nitrogen cycling following mountain pine beetle disturbance in lodgepole pine forests of Greater Yellowstone. Forest Ecology and Management 261(6): 1077-1089.

Hancock JE, Arthur MA, Weathers KC, Lovett GM. 2008. Carbon cycling along a gradient of beech bark disease impact in the Catskill Mountains, New York. Canadian Journal of Forest Research 38(5): 1267-1274.

Hansen EM. 2015. Phytophthora Species Emerging as Pathogens of Forest Trees. Current Forestry Reports 1(1): 16-24.

Hicke JA, Allen CD, Desai AR, Dietze MC, Hall RJ, Hogg EH, Kashian DM, Moore D, Raffa KF, Sturrock RN, Vogelmann J. 2012. Effects of biotic disturbances on forest carbon cycling in the United States and Canada. Global Change Biology 18(1): 7-34.

Hinsinger P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil 237(2): 173-195.

Hobara S, Tokuchi N, Ohte N, Koba K, Katsuyama M, Kim SJ, Nakanishi A. 2001. Mechanism of nitrate loss from a forested catchment following a small-scale, natural disturbance. Canadian Journal of Forest Research 31(8): 1326-1335.

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Hogberg MN, Nyberg G, Ottosson-Lofvenius M, Read DJ. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411(6839): 789-792.

Högberg P, Read DJ. 2006. Towards a more plant physiological perspective on soil ecology. Trends in Ecology & Evolution 21(10): 548-554.

Hubbell SP, Ahumada JA, Condit R, Foster RB. 2001. Local neighborhood effects on long-term survival of individual trees in a neotropical forest. Ecological Research 16(5): 859-875.

Ibáñez B, Gómez-Aparicio L, Stoll P, Ávila JM, Pérez-Ramos IM, Marañón T. 2015. A neighborhood analysis of the consequences of Quercus suber decline for regeneration dynamics in Mediterranean forests. PloS one 10(2): e0117827.

Jackson L, Strauss R, Firestone M, Bartolome J. 1988. Plant and soil nitrogen dynamics in California annual grassland. Plant and Soil 110(1): 9-17.

Johnson JB, Omland KS. 2004. Model selection in ecology and evolution. Trends in Ecology and Evolution 19(2): 101-108.

Joshi M, Mer GS, Singh SP, Rawat YS. 1991. Seasonal pattern of total soil respiration in undisturbed and disturbed ecosystems of Central Himalaya. Biology and Fertility of Soils 11(4): 267-272.

Jurinak JJ, Dudley LM, Allen MF, Knight WG. 1986. The role of calcium-oxalate in the availability of phosphorus in soils of semiarid regions - a thermodynamic study. Soil Science 142(5): 255-261.

Keith H, Wong SC. 2006. Measurement of soil CO2 efflux using soda lime absorption: both quantitative and reliable. Soil Biology & Biochemistry 38(5): 1121-1131.

Kizlinski ML, Orwig DA, Cobb RC, Foster DR. 2002. Direct and indirect ecosystem consequences of an invasive pest on forests dominated by eastern hemlock. Journal of Biogeography 29(10-11): 1489-1503.

Kurz WA, Dymond CC, Stinson G, Rampley GJ, Neilson ET, Carroll AL, Ebata T, Safranyik L. 2008. Mountain pine beetle and forest carbon feedback to climate change. Nature 452(7190): 987-990.

Legendre P, Fortin MJ. 1989. Spatial pattern and ecological analysis. Vegetatio 80(2): 107-138.

Levy-Varon JH, Schuster WS, Griffin KL. 2012. The autotrophic contribution to soil respiration in a northern temperate deciduous forest and its response to stand disturbance. Oecologia 169(1): 211-220.

Levy-Varon JH, Schuster WSF, Griffin KL. 2014. Rapid rebound of soil respiration following partial stand disturbance by tree girdling in a temperate deciduous forest. Oecologia 174(4): 1415-1424.

Loo JA. 2009. Ecological impacts of non-indigenous invasive fungi as forest pathogens. Biological Invasions 11(1): 81-96.

Lovett GM, Arthur MA, Weathers KC, Griffin JM. 2010. Long-term changes in forest carbon and nitrogen cycling caused by an introduced pest/pathogen complex. Ecosystems 13(8): 1188-1200.

Lovett GM, Canham CD, Arthur MA, Weathers KC, Fitzhugh RD. 2006. Forest ecosystem responses to exotic pests and pathogens in eastern North America. Bioscience 56(5): 395-405.

Ludwig F, de Kroon H, Berendse F, Prins HH. 2004. The influence of savanna trees on nutrient, water and light availability and the understorey vegetation. Plant Ecology 170(1): 93-105.

Lloret F, Mattana S, Curiel Yuste J. 2015. Climate-induced die-off affects plant–soil–microbe ecological relationship and functioning. FEMS Microbiology Ecology 91(2): 1-12.

Martínez-Vilalta J, Lloret F, Breshears DD. 2012. Drought-induced forest decline: causes, scope and implications. Biology Letters 8(5): 689-691.

Martínez F, Lazo Y, Fernández-Galiano J, Merino J. 2002. Root respiration and associated costs in deciduous and evergreen species of Quercus. Plant, Cell & Environment 25(10): 1271-1278.

McDowell NG, Ryan MG, Zeppel MJB, Tissue DT. 2013. Improving our knowledge of drought-induced forest mortality through experiments, observations, and modeling. New Phytologist 200(2): 289-293.

Mladenoff DJ. 1987. Dynamics of nitrogen mineralization and nitrification in hemlock and hardwood treefall gaps. Ecology 68(5): 1171-1180.

Morehouse K, Johns T, Kaye J, Kaye A. 2008. Carbon and nitrogen cycling immediately following bark beetle outbreaks in southwestern ponderosa pine forests. Forest Ecology and Management 255(7): 2698-2708.

Morillas L, Gallardo A, Portillo-Estrada M, Covelo F. 2012. Nutritional status of Quercus suber populations under contrasting tree dieback. Forestry 85(3): 369-378.

Murphy L 2012. Likelihood: Methods for maximum likelihood estimation. R package version 1.6.

Nave LE, Gough CM, Maurer KD, Bohrer G, Hardiman BS, Le Moine J, Munoz AB, Nadelhoffer KJ, Sparks JP, Strahm BD, Vogel CS, Curtis PS. 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. Journal of Geophysical Research-Biogeosciences 116: G04016.

Ninyerola M, Pons X, Roure JM. 2005. Atlas climático digital de la Península Ibérica: metodología y aplicaciones en bioclimatología y geobotánica. Barcelona: Universidad Autónoma de Barcelona.

Nuckolls AE, Wurzburger N, Ford CR, Hendrick RL, Vose JM, Kloeppel BD. 2009. Hemlock declines rapidly with hemlock woolly adelgid infestation: impacts on the carbon cycle of Southern Appalachian forests. Ecosystems 12(2): 179-190.

Ojeda F, Marañón T, Arroyo J. 2000. Plant diversity patterns in the Aljibe Mountains (S. Spain): A comprehensive account. Biodiversity and Conservation 9(9): 1323-1343.

Orwig DA, Cobb RC, D'Amato AW, Kizlinski ML, Foster DR. 2008. Multi-year ecosystem response to hemlock woolly adelgid infestation in southern New England forests. Canadian Journal of Forest Research 38(4): 834-843.

Pumpanen J, Longdoz B, Kutsch WL 2010. Field measurements of soil respiration: principles and constraints, potentials and limitations of different methods. In: Kutsch WL, Bahn M, Heinemeyer A eds. Soil Carbon Dynamics. New York, USA: Cambridge University Press, 16-33.

Qian P, Schoenau JJ. 2002. Practical applications of ion exchange resins in agricultural and environmental soil research. Canadian Journal of Soil Science 82(1): 9-21.

R Core Team 2013. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rodríguez A, Durán J, Fernández-Palacios JM, Gallardo A. 2009. Wildfire changes the spatial pattern of soil nutrient availability in Pinus canariensis forests. Annals of Forest Science 66(2): 1-7.

Rodríguez Pleguezuelo CR, Durán Zuazo VH, Muriel Fernández JL, Martín Peinado FJ, Franco Tarifa D. 2009. Litter decomposition and nitrogen release in a sloping Mediterranean subtropical agroecosystem on the coast of Granada (SE, Spain): Effects of floristic and topographic alteration on the slope. Agriculture, Ecosystems and Environment 134(1-2): 79-88.

Sardans J, Peñuelas J. 2005. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. forest. Soil Biology & Biochemistry 37(3): 455-461.

Sardans J, Rodà F, Peñuelas J. 2004. Phosphorus limitation and competitive capacities of Pinus halepensis and Quercus ilex subsp. rotundifolia on different soils. Plant Ecology 174(2): 305-317.

Schneider K, Turrión MB, Grierson PF, Gallardo JF. 2001. Phosphatase activity, microbial phosphorus, and fine root growth in forest soils in the Sierra de Gata, western central Spain. Biology and Fertility of Soils 34(3): 151-155.

Schoenau JJ, Huang WZ. 1991. Anion-exchange membrane, water, and sodium-bicarbonate extractions as soil tests for phosphorus. Communications in Soil Science and Plant Analysis 22(5-6): 465-492.

Shearer BL, Crane CE, Fairman RG, Dunne CP. 2009. Ecosystem dynamics altered by pathogen-mediated changes following invasion of Banksia woodland and Eucalyptus marginata forest biomes of south-western Australia by Phytophthora cinnamomi. Australasian Plant Pathology 38(4): 417-436.

Sheriff D, Nambiar E, Fife D. 1986. Relationships between nutrient status, carbon assimilation and water use efficiency in Pinus radiata (D. Don) needles. Tree Physiology 2(1-2-3): 73-88.

Sims GK, Ellsworth TR, Mulvaney RL. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. Communications in Soil Science and Plant Analysis 26(1-2): 303-316.

Singh JS, Gupta SR. 1977. Plant decomposition and soil respiration in terrestrial ecosystems. The Botanical Review 43(4): 449-528.

Søe AR, Buchmann N. 2005. Spatial and temporal variations in soil respiration in relation to stand structure and soil parameters in an unmanaged beech forest. Tree Physiology 25(11): 1427-1436.

Sturrock R, Frankel S, Brown A, Hennon P, Kliejunas J, Lewis K, Worrall J, Woods A. 2011. Climate change and forest diseases. Plant Pathology 60(1): 133-149.

Tang JW, Baldocchi DD. 2005. Spatial-temporal variation in soil respiration in an oak-grass savanna ecosystem in California and its partitioning into autotrophic and heterotrophic components. Biogeochemistry 73(1): 183-207.

Urbieta IR, Zavala MA, Marañón T. 2008. Human and non-human determinants of forest composition in southern Spain: Evidence of shifts towards cork oak dominance as a result of management over the past century. Journal of Biogeography 35(9): 1688-1700.

Uriarte M, Condit R, Canham CD, Hubbell SP. 2004. A spatially explicit model of sapling growth in a tropical forest: Does the identity of neighbours matter? Journal of Ecology 92(2): 348-360.

van Mantgem PJ, Stephenson NL, Byrne JC, Daniels LD, Franklin JF, Fule PZ, Harmon ME, Larson AJ, Smith JM, Taylor AH, Veblen TT. 2009. Widespread increase of tree mortality rates in the western United States. Science 323(5913): 521-524.

Wang WF, Peng CH, Kneeshaw DD, Larocque GR, Luo ZB. 2012. Drought-induced tree mortality: ecological consequences, causes, and modeling. Environmental Reviews 20(2): 109-121.

Weste G, Marks GC. 1987. The biology of Phytophthora cinnamomi in australasian forests. Annual Review of Phytopathology 25: 207-229.

Xiong Y, D'Atri JJ, Fu S, Xia H, Seastedt TR. 2011. Rapid soil organic matter loss from forest dieback in a subalpine coniferous ecosystem. Soil Biology and Biochemistry 43(12): 2450-2456.

Zhang QH, Zak JC. 1995. Effects of gap size on litter decomposition and microbial activity in a subtropical forest. Ecology 76(7): 2196-2204.

Ziadi N, Simard RR, Allard G, Lafond J. 1999. Field evaluation of anion exchange membranes as a N soil testing method for grasslands. Canadian Journal of Soil Science 79(2): 281-294.

Supporting information

Table S1 Results of the Mantel test analysing the spatial autocorrelation in the residuals of the models. Mantel tests based on 9999 replications.

Type of forest	Soil variable	Season	r	p-value
Woodlands	CO_2	SPR	-0.010	0.685
		SUM	-0.035	0.962
	NH_4^+	SPR	-0.082	1.000
		SUM	-0.075	1.000
	NO_3^-	SPR	-0.013	0.718
		SUM	-0.048	1.000
	PO_4^{3-}	SPR	0.008	0.324
		SUM	-0.051	1.000
Closed forests	CO_2	SPR	0.087	0.008
		SUM	-0.032	0.895
	NH_4^+	SPR	-0.019	0.825
		SUM	0.002	0.413
	NO_3^-	SPR	-0.076	1.000
		SUM	0.021	0.104
	PO_4^{3-}	SPR	0.017	0.168
		SUM	0.103	<0.001

Figure S1 Model residuals against time.

Table S2 Parameter estimates and 2-unit support intervals (in brackets) for the best model selected for each combination of soil variables and seasons in woodlands. Values of coefficients $a_{site\text{-}year}$, b_{year} are given. Different values of $a_{site\text{-}year}$ coefficient indicate differences in background levels for different sites and year combinations (S1 = site 1, S2 = site 2, S3 = site 3). Different values of b_{year} coefficient indicate a different effect in the slope of tree effects (‡: No differences were found between years). Parameters α, β and λ of the neighbourhood index (NI – Eq. 3) are given (see text for details). NA: year without data for that variable.

Parameters	CO_2		NH_4^+		NO_3^-		PO_4^{3-}	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
$a_{Site\text{-}Year}$ S1-2010	NA	NA	4.22 [3.72,4.98]	8.17 [5.80,8.83]	9.00 [7.20,10.82]		3.08 [2.25,3.34]	1.01 [0.83,1.20]
S1-2011	502.5 [467.3,545.5]	245.7 [206.4,268.2]	1.07 [0.84,1.33]	5.67 [4.76,6.37]	4.06 [2.76,4.89]	20.49‡ [17.83,24.69]	0.87 [0.76,1.04]	1.44 [1.20,1.70]
S1-2012	420.2 [399.2,481.9]	188.8 [137.2,184.6]	NA	9.23 [8.58,12.49]	NA		2.11 [1.89,2.69]	4.37 [3.45,5.07]
S2-2010	NA	NA	10.73 [9.44,12.39]	4.12 [3.63,5.63]	13.30 [9.58,15.77]		9.83 [8.75,11.73]	2.54 [1.98,2.93]
S2-2011	655.6 [622.8,718.3]	686.6 [604.2, 762.1]	5.39 [4.52,6.19]	9.00 [7.02,10.54]	6.07 [5.03,10.02]	71.08‡ [63.26,79,74]	3.22 [2.81,3.84]	3.73 [3.43,5.23]
S2-2012	577.8 [485.6,565.7]	137.5 [119.6,170.2]	NA	17.31 [15.75, 21.23]	NA		4.52 [3.89,5.28]	5.91 [4.61,6.72]
S3-2010	NA	NA	2.80 [1.93,3.17]	2.04 [1.71, 2.62]	0.29 [0.00,1.80]		7.43 [6.09,8.54]	0.64 [0.57,0.82]
S3-2011	597.8 [549.9,641.3]	536.7 [456.2,583.1]	0.97 [0.67,1.32]	1.73 [1.45, 2.74]	1.54 [0.14,3.43]	27.66‡ [24.06,33.03]	1.45 [1.30,1.80]	1.95 [1.62,2.30]
S3-2012	459.2 [427.0,511.1]	197.9 [158.3,217.9]	NA	4.25 [3.70, 5.30]	NA		3.27 [2.78,3.76]	0.35 [0.31,0.44]
b_{Year} 2010				16.52 [12.33, 20.86]				
2011	2.24‡ [1.04, 4.95]	19.93‡ [15.85,22.32]	0.84‡ [0.70,0.94]	29.79 [24.60, 36.01]	1.64‡ [1.48,1.84]	194.06‡ [130.03,233.29]	1.99‡ [1.61,2.41]	0.64‡ [0.58,0.73]
2012				4.71 [2.57, 13.86]				
$\lambda_{heterospecific}$	0.72 [-0.09,1.00]	0.80 [0.18,1.00]	0.62 [0.36,0.84]	-0.03 [-0.04,0.26]	0.37 [0.13,0.59]	-0.53 [-0.68,-0.21]	-0.42 [-0.59,-0.30]	0.97 [0.52,1.00]
$\lambda_{Q.\ suber}$	0.70 [0.21,1.00]		0.35 [0.28,0.41]		0.66 [0.61,0.75]		-0.09 [-0.12,0.05]	
$\lambda_{Q.\ suber\ healthy}$		1.00 [0.78,1.00]		0.91 [0.79,1.00]		0.93 [0.61,1.00]		0.90 [0.79,1.00]
$\lambda_{Q.\ suber\ defoliated}$		0.10 [-0.25,0.27]		0.03 [-0.02,0.13]		0.36 [0.03,0.58]		0.31 [0.18,0.55]
$\lambda_{Q.\ suber\ dead}$		1.00 [-0.09,1.00]		0.05 [-0.04,0.17]		0.49 [-0.22,1.00]		-0.02 [-0.24,0.34]
α	3.94 [3.27,4.00]	0.01 [0.00,0.17]	1.25 [1.16,1.42]	0.62 [0.48, 0.74]	0.14 [0.03,0.21]	3.62 [3.22,4.00]	1.49 [1.27, 1.74]	0.65 [0.52,0.73]
β	2.64 [1.90,3.35]	0.41 [0.36,0.53]	0.00 [0.00,0.07]	1.79 [1.63, 1.86]	0.35 [0.29,0.39]	0.98 [0.88,1.15]	0.69 [0.60, 0.81]	0.96 [0.88,1.00]

Table S3 Parameter estimates and 2-unit support intervals (in brackets) for the best model selected for each combination of soil variables and seasons in closed forests. Values of coefficients $a_{site\text{-}year}$, b_{year} are given. Different values of $a_{site\text{-}year}$ coefficient indicate differences in background levels for different sites and year combinations (S1 = site 1, S2 = site 2, S3 = site 3). Different values of b_{year} coefficient indicate a different effect in the slope of tree effects (‡: No differences were found between years). Parameters α, β and λ of the neighbourhood index (NI – Eq. 3) are given (see text for details). NA: year without data for that variable.

Parameters	CO_2		NH_4^+		NO_3^-		PO_4^{3-}	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
$a_{Site\text{-}Year}$ S1-2010	NA	NA	9.36 [8.80,10.99]	25.82 [22.47,33.50]	36.47 [35.01,39.76]	36.33 [34.87,41.78]	1.43 [1.00,1.75]	0.26 [0.23,0.37]
S1-2011	499.4 [464.5,537.5]	416.9 [375.2,436.9]	28.63 [23.76,32.91]	10.11 [6.57,17.94]	49.44 [45.98, 50.00]	34.06 [33.72,37.38]	1.27 [0.90,1.67]	2.08 [1.81,2.51]
S1-2012	503.6 [473.4,541.6]	299.9 [281.9,322.9]	6.02 [5.96,6.08]	40.16 [29.31,42.55]	NA	49.42 [39.54,52.95]	0.43 [0.25,1.25]	0.01 [0.00,0.02]
S2-2010	NA	NA	4.88 [4.34,5.69]	8.93 [2.05,12.03]	14.07 [13.50,16.22]	21.41 [17.56,23.62]	2.57 [2.13,2.90]	0.61 [0.57,1.05]
S2-2011	600.4 [552.4,644.1]	364.9 [335.7,384.9]	3.46 [3.15,3.99]	21.17 [14.82,25.17]	15.91 [13.84,16.75]	25.68 [22.85,28.91]	0.80 [0.54,1.32]	1.33 [1.33,1.34]
S2-2012	574.4 [539.9,617,8]	168.4 [151.6,176.9]	7.51 [5.85,8.32]	34.85 [31.71,42.67]	NA	38.12 [35.45,48.40]	0.55 [0.27,1.06]	2.44 [1.02,2.82]
S3-2010	NA	NA	4.36 [3.93,5.21]	11.81 [1.30,14.28]	12.11 [12.11,12.11]	39.60 [37.62,46.85]	2.24 [1.84,2.60]	0.76 [0.63,0.99]
S3-2011	897.0 [816.3,942.4]	685.6 [603.3,726.7]	10.77 [8.72,12.30]	17.53 [13.67,24.30]	16.03 [15.23,17.03]	56.51 [50.86,64.04]	0.74 [0.37,1.11]	6.15 [5.66,7.55]
S3-2012	616.9 [555.2,716.9]	359.9 [334.7,385.5]	5.36 [4.39,5.98]	32.89 [24.66,35.90]	NA	44.46 [39.12,48.24]	1.56 [1.18,1.92]	1.07 [0.92,3.00]
b_{Year} 2010				-401.87 [-1296.95,754.40]				1.31 [1.13,2.97]
2011		2059.0‡ [1100.8, 3063.5]	0.45‡ [0.45,0.46]	-722.82 [-1372.23,871.38]	2.05‡ [2.05,2.05]	11.76‡ [10.55,11.96]		-3.70 [-3.75,-3.61]
2012				3015.2 [2092.6,4211.1]				16.41 [15.19,20.35]
$\lambda_{heterospecific}$		0.85 [0.43,1.00]	0.19 [0.10,0.25]	-0.26 [-0.44,-0.04]	-0.65 [-0.62,-0.61]	-0.68 [-0.70,-0.60]		0.40 [0.40,0.40]
$\lambda_{Q.\ suber}$								
$\lambda_{Q.\ suber\ healthy}$		0.24 [0.01,0.70]	-0.93 [-0.95,-0.89]	0.02 [-0.04,0.08]	-0.99 [-1.00,-0.98]	-0.73 [-0.84,-0.60]		0.99 [0.93,1.00]
$\lambda_{Q.\ suber\ defoliated}$		0.57 [-0.12,0.82]	-0.99 [-1.00,-0.93]	0.09 [-0.03,0.16]	-0.99 [-1.00,-0.98]	0.49 [0.31,0.71]		0.09 [0.08,0.11]
$\lambda_{Q.\ suber\ dead}$		-0.97 [-1.00,0.40]	0.05 [0.03,0.09]	0.99 [0.66,1.00]	0.64 [0.56,0.77]	0.72 [0.59, 1.00]		-0.05[-0.06, -0.04]
α		3.67 [3.19, 4.00]	0.00 [0.00, 0.02]	3.99 [3.88,4.00]	0.70 [0.70,0.70]	1.47 [1.47,1.57]		3.95 [3.92,3.99]
β		2.66 [2.20, 3.38]	0.57 [0.57,0.58]	3.98 [3.66,4.00]	0.00 [0.00,0.00]	0.22 [0.21,0.27]		0.37 [0.37,0.38]

PATHOGEN-INDUCED TREE MORTALITY MODIFIES KEY COMPONENTS OF THE C AND N CYCLES WITH NO CHANGES ON MICROBIAL FUNCTIONAL DIVERSITY

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Pathogen-induced tree mortality modifies key components of the C and N cycles with no changes on microbial functional diversity

Resumen

En las últimas décadas se ha detectado un incremento en la mortalidad arbórea como consecuencia de alteraciones bióticas relacionadas con el cambio global en ecosistemas a escala mundial. Las alteraciones bióticas en los bosques podrían causar impactos significativos en la actividad biológica del suelo y en la transformación de la materia orgánica. Sin embargo, hasta la fecha muy pocos estudios empíricos han explorado las consecuencias de alteraciones bióticas para las comunidades microbianas del suelo y los ciclos del carbono (C) y nitrógeno (N). En este trabajo se han analizado los efectos del decaimiento de *Quercus suber* en la diversidad funcional, la biomasa y la actividad de la comunidad microbiana en bosques mixtos mediterráneos invadidos por el patógeno de raíces exótico *Phytophthora cinnamomi*, y se han conectado estos efectos con alteraciones de componentes clave de los ciclos del C y N. El estudio se replicó en los dos principales tipos de bosques de *Q. suber* de la región (bosque abierto y bosque cerrado) con diferencias contrastadas en la textura del suelo. Se utilizaron modelos de vecindad espacialmente explícitos para analizar los efectos directos del decaimiento de *Q. suber* en las variables del suelo (comparando el impacto de árboles de *Q. suber* con diferente estado de salud), así como su potencial efecto indirecto a largo plazo (comparando el impacto de individuos sanos de *Q. suber* con individuos de las especies coexistentes no afectadas por decaimiento *Olea europea* var. *Sylvestris* y *Quercus canariensis*). El decaimiento inducido por patógeno no afectó a la diversidad funcional ni a la biomasa microbiana, pero sí provocó una menor respiración microbiana en el suelo. La mortalidad de árboles también provocó cambios en variables de los ciclos del C y el N (p. ej. C total del suelo, C orgánico disuelto, hexosas, pentosas, nitrógeno inorgánico del suelo), aunque el signo y la magnitud de estos cambios varió dependiendo de las características locales de la textura del suelo. Se detectaron diferencias importantes entre el efecto de las especies coexistentes y el de *Q. suber* sobre los ciclos del C y el N. En general, los resultados mostraron que el decaimiento asociado a patógenos invasores provoca efectos complejos a corto y largo plazo en diferentes componentes de los ciclos del C y el N, sin afectar a la diversidad funcional ni la biomasa microbiana.

Abstract

In the last decades, an increase in tree mortality rates as a consequence of global change-related biotic disturbances has been detected worldwide. Biotic disturbances in forests might cause significant impacts on soil biological activity and organic matter transformation. However, very few empirical studies have explored to date the consequences of biotic disturbances for soil microbial communities and carbon (C) and nitrogen (N) cycles. Here we assessed the effects of *Quercus suber* dieback on the functional diversity, biomass and activity of the soil microbial community in mixed Mediterranean forests invaded by the exotic root pathogen *Phytophthora cinnamomi,* and connected these effects with alterations of key components of the C and N cycles. The study was replicated in the two main *Q. suber* forest types of the region (closed forests and open woodlands) with contrasting texture characteristics. We used a spatially-explicit neighbourhood approach to explore the direct effects of *Q. suber* dieback on soil variables (by comparing the impact of *Q. suber* trees with different health status) as well as its potential long-term indirect effects (comparing the impact of healthy *Q. suber* with non-declining coexistent tree species *Olea europaea* var. *sylvestris* and *Quercus canariensis*). Pathogen-induced tree dieback did not affect microbial functional diversity or biomass, but translated into lower soil microbial respiration. Tree mortality also induced changes in several variables of the C and N cycle (e.g. total soil C, DOC, hexoses, pentoses, soil inorganic N), but the sign and magnitude of these effects varied depending on the local characteristics of soil texture. Coexistent species differed strongly from *Q. suber* in their effects on the C and N cycle. Overall, our results show that tree dieback due to invasive pathogens translates into complex short- and long-term effects on different components of the C and N cycles, despite no effects on microbial functional diversity and biomass.

Keywords

Microbial functional diversity, tree mortality, microbial respiration, microbial biomass, organic matter, nitrogen

Introduction

During the last decade there has been a growing awareness about an increase in tree mortality rates worldwide driven by biotic and abiotic factors related to global change (Allen *et al.*, 2010; Allen *et al.*, 2015; Trumbore *et al.*, 2015; Lovett *et al.*, 2016). Tree mortality can substantially alter belowground ecosystem processes as a consequence of the tight relationship existent between the tree community and soil processes (e.g. Wardle *et al.*, 2004; Van Der Putten *et al.*, 2009). The characteristics of the plant community determine the quantity and quality of C inputs for the soil community (i.e. herbivores, decomposers, mutualists…). In turn, soil organisms regulate the aboveground community composition and growth directly by establishing associations with plant roots and indirectly by releasing nutrients during organic matter decomposition (Bardgett & Wardle, 2010). In a global change context where tree communities are being altered by multiple factors such as warming and drought, wildfires, or exotic species, there is a pressing need to understand how these changes could translate into altered functioning of the belowground subsystem (Wardle & Peltzer, 2017).

In order to understand the implications of tree mortality for the soil biological activity, it becomes fundamental to assess its impacts on the microbial community and soil organic matter. These key components of the soil influence the whole soil food web and therefore greatly impact nutrient cycles and many biologically related soil properties (Weil *et al.*, 2003; Schlesinger & Bernhardt, 2012). Furthermore, analyzing the effect of tree mortality on microbial diversity and soil functioning might provide valuable insights into the ongoing debate about the biodiversity-ecosystem functioning (BEF) relationship. Several studies have found that a reduction in taxonomic soil diversity as a consequence of global change is directly related to a reduction in soil functioning (e.g. Allison & Martiny, 2008; Bell *et al.*, 2009; Delgado-Baquerizo *et al.*, 2016a). However, obtaining a general or predictable pattern is complex, since different factors such as soil fertility or the identity of the function studied can condition the level of functional redundancy in microbial communities and consequently BEF relationships (Hättenschwiler *et al.*, 2005; Van Der Heijden *et al.*, 2008; Delgado-Baquerizo *et al.*, 2016b). Our understanding of the BEF relationship in the soil subsystem would very much benefit from additional studies that explore the link between different forms of diversity (not only taxonomic) and soil functioning in different global change scenarios (Bardgett & van der Putten, 2014).

Recent studies have shown that biotic disturbances such as pests or pathogens usually lead to significant alterations of plant communities that might result in cascading impacts on belowground communities and processes (e.g. Edburg *et al.*, 2012; Hicke *et al.*, 2012; Lovett *et al.*, 2016). While little is known about the effects of biotic disturbances on functional diversity and biomass of the microbial community, previous studies have found profound effects on stocks and cycling rates of C and N. For instance, a recent meta-analysis showed that biotic disturbances significantly decrease below-ground C pools with relatively long residence times, but increase labile C pools with short residence times (Zhang *et al.*, 2015). Moreover, previous studies found an increase in soil organic and inorganic N in stands affected by insect or pathogen-related tree mortality (e.g. Hobara *et al.*, 2001; Stadler *et al.*, 2005; Morehouse *et al.*, 2008; Edburg *et al.*, 2012). Besides the short-term effects of biotic disturbances, in the long term (i.e. several decades after the onset of the disturbance), impacts on belowground processes might occur as a consequence of species replacements. This phenomenon is more likely to happen when the affected species is functionally different from coexistent non-affected species (e.g. Hancock *et al.*, 2008; Lovett *et al.*, 2010; Cobb *et al.*, 2013). Despite an urgent need for a better understanding of the short- and long-term impacts of biotic disturbances on the belowground system, very few empirical studies have yet explored the consequences of forest pests and pathogens for soil microbial communities and soil C and N dynamics (Hicke *et al.*, 2012; Holden & Treseder, 2013; Zhang *et al.*, 2015), which preclude us from making accurate predictions of the implications of these increasing disturbances for soil biology.

In southern Europe, a severe decline of *Quercus suber* and *Quercus ilex* has been detected since the early 1990's. This phenomenon is considered an important concern for managers, landowners and researchers due to the economic and ecological importance of these tree species in the region. The decline has been related to the attack by the exotic soil-borne pathogen *Phytophthora cinnamomi* (e.g. Brasier, 1992; Sánchez *et al.*, 2002; Camilo-Alves *et al.*, 2013), which is considered one of the 100 most dangerous invasive species on earth (Weste & Marks, 1987; Lowe *et al.*, 2000). This biotic disturbance results in important changes in forest composition and structure, with a reduction in tree cover of the dominant evergreen oak species and an increase in abundance of coexistent species not affected by the pathogen-induced mortality (Camilo-Alves *et al.*, 2013; Ávila *et al.*, 2017). In this study we aim to assess the impact of the decline of the key tree species *Quercus suber* on the soil microbial community and the C and N cycle in Mediterranean

forests heavily invaded by *P. cinnamomi*. Specifically, we measured the functional diversity, biomass (C and N) and activity (respiration) of the microbial community. We also measured different components of the C cycle (i.e. total soil C, dissolved organic carbon [DOC] and significant components of the dissolved organic matter such as hexoses, pentoses, amino acids and phenols) and the N cycle (i.e. dissolved organic nitrogen [DON], NH_4^+ and NO_3^-). This study was conducted covering an extensive spatial area in two contrasting forest types representative of the main mixed *Q. suber* forest types of the region: open woodlands and closed forests. We performed spatially-explicit neighborhood models to explore: 1) the direct effects of pathogen-induced *Q. suber* decline by comparing the impact on soil variables of *Q. suber* trees with different health status (i.e. healthy, defoliated and dead), and 2) the potential long-term indirect effects by comparing the impact of (healthy) *Q. suber* trees and of coexistent species not affected by defoliation and mortality (i.e. *Olea europaea* var. *sylvestris* and *Quercus canariensis*).

We tested five specific hypotheses. First, we predicted that pathogen-induced mortality would cause a decrease in microbial functional diversity due to reductions in the diversity of C compounds reaching the soil as a consequence of tree decline (Cai *et al.*, 2010; Rodríguez *et al.*, 2016), but an increase in microbial biomass and respiration lead by an increase in C mineralization as reported for other biotic disturbances (Edburg *et al.*, 2012; Zhang *et al.*, 2015). Second, we expected that tree mortality would increase C sources with low residence times (e.g. DOC, hexoses, pentoses) but would decrease C pools with high residence times (e.g. total soil C, phenols) also as a consequence of reductions in C inputs to the soil and increments in C mineralization (Hicke *et al.*, 2012; Zhang *et al.*, 2015). Third, we predicted that pathogen-induced tree mortality would cause an increase in organic and inorganic N due to litterfall, reductions in N uptake and favorable conditions for mineralization (e.g. Stadler *et al.*, 2005; Morehouse *et al.*, 2008; Edburg *et al.*, 2012). Fourth, as a consequence of the key role that texture have on C and N dynamics (e.g. Allison, 1973; Hassink, 1997; Hassink & Whitmore, 1997; Müller & Höper, 2004), we expected different impacts of pathogen-induced mortality in the two forest types of study due to their differences in soil texture, clay content being much higher in open woodlands than in closed forests (Gómez-Aparicio *et al.*, 2012). Fifth, we predicted different 'footprints' of coexistent species on the studied soil variables given their differences in leaf traits (e.g. Aponte *et al.*, 2011), which could lead to long-term indirect impacts of *Q. suber* mortality on ecosystem function due to its substitution by coexistent woody species.

Materials and methods

Study site

This study was carried out in mixed oak forests in Los Alcornocales Natural Park (Southern Spain), considered one of the largest *Quercus suber* L. forests within the Mediterranean Basin (Urbieta *et al.*, 2008). The climate in these forests is classified as sub-humid Mediterranean, and characterized by mild and humid winters and warm and dry summers. Annual mean temperature ranges from 15.4 to 17.3 ºC and annual mean rainfall varies from 720 to 1100 mm (period 1951-1999, Ninyerola *et al.*, 2005). These forests occur on acidic, nutrient-poor, sandy soils, derived from Oligo-Miocene sandstone, but sometimes they are interspersed with clay layers derived from marl sediments. The flora in Los Alcornocales Natural Park is dominated by *Q. suber* as the main species. Trees of this dominant species form two different systems in the Natural Park. In the drier lowlands of the park, where sandy soils are interspersed with clay layers, *Q. suber* coexists with the evergreen and shade-intolerant *Olea europaea* var. *sylvestris* Brot. forming open woodlands, while in moister habitats with sandy soils *Q. suber* is intermingled with the deciduous shade-tolerant *Quercus canariensis* Willd. forming closed forests (Ojeda *et al.*, 2000).

In the study area, *Q. suber* adult trees show important problems of defoliation and mortality. Extremely high abundances of *Phytophthora cinnamomi* Rands have been found in soils of symptomatic *Q. suber* trees (Gómez-Aparicio *et al.*, 2012). Therefore, this pathogen has been suggested as a main driver of the species decline in the area (Sánchez *et al.*, 2002). No other tree species or shrubs in the study area showed evident symptoms of dieback.

Field sampling and neighborhood characterization

This study was conducted in 6 sites within the Natural Park three of them located in open woodlands (i.e. mixed forests of *Q. suber* and *O. europaea*) and the other three in closed forests (i.e. mixed forests of *Q. suber* and *Q. canariensis*). Soils in the open woodland sites are characterized by a higher clay content than soils in the closed forest sites (Supporting Information Tables S1). At each site, we established a permanent 70 x 70 m^2 plot that was subdivided into a grid with 49 10 x 10 m^2 subplots. At the center of each subplot we established a sampling point, with 147 points per forest type (a total of 294).

Soils for this study were collected from each sampling point during Spring 2012. Samples were taken from the top 15 cm using a metal corer (5 cm in diameter). Finally, soils were transported cold to the laboratory, where they were sieved (2-mm mesh) and kept at 4ºC until analyses. For microbial analyses, a subsample of soils was air-dried at room temperature for four weeks, until constant weight.

To characterize local neighborhoods, we mapped and identified all live and dead trees around each sampling point. We determined a neighborhood as a 15-m radius circle around each sampling point (Gómez-Aparicio *et al.*, 2012; Ávila *et al.*, 2016). In each neighborhood we identified and mapped all trees higher than 1.5 m and with a diameter at breast height (d.b.h.) > 2 cm using a total station Leica TC 407 (Leica Geosystems, Heerbrugg, Switzerland). We measured the d.b.h. of each of the trees mapped (n = 1341 trees). In addition, we evaluated the health status of *Q. suber* trees by a visual estimation of crown defoliation: healthy reference trees (without signs of crown defoliation), defoliated trees, and dead trees (standing trees with no leaves).

Analysis of the microbial community: functional diversity, microbial biomass and respiration

We estimated the community level physiological profile (CLPP) using the MicroResp™ soil respiration system (Campbell *et al.*, 2003). This is a technique based on the analyses of soil heterotrophic microbial community activity exposed to 15 different carbon sources that vary in structural complexity. We selected amino acids (AA: L-alanine, L-arginine, L-cysteine, L-lysine and N-acetyl-glucosamine), carbohydrates (CH: D-fructose, D-galactose, D-glucose, D-trehalose, L-arabinose) and carboxylic acids (CA: amino butyric acid, oxoglutaric acid, malic acid and oxalic acid) that has been successfully used in previous studies using MicroResp™ (e.g. Oren & Steinberger, 2008; García-Palacios *et al.*, 2011; Delgado-Baquerizo *et al.*, 2013). This technique is commonly used to detect changes in heterotrophic functional diversity because these C-compounds correspond to the catabolic attributes of diverse soil microbial functional groups (Zak *et al.*, 1994). Soil subsamples were introduced into flasks and pre-incubated for 5 days at 25ºC and with the moisture adjusted to 40% of their water holding capacity (WHC) in order to condition the soils and reestablish active microbial populations. A final volume of 400 mL of soil per well was placed in 96-well microtiter deep well plates. Three lab replicates were used per each field sample. C-compounds were added to soils at a concentration of 30 mg C g soil water^{-1}. Previously, soil water content was determined gravimetrically and the quantity

of C source to be added was calculated to have final water contents of approximately 50% WHC during the assay. We determined the respiration induced by the C-compounds after 6 h of incubation (25ºC) by absorbance measurements (at 570 nm) in detector plates, consisting of microplates filled with cresol red agar. Induced respiration was reported as means of three lab replicates from each field soil sample. The results were calculated on the basis of water, which represents the basal respiration of the microbial community (hereafter, microbial respiration, R_{mic}). This basis was measured simultaneously in wells filled with soils at the same water content but without C-compound addition. A microbial catabolic profile was created in the form of a data matrix of the respiration induced by the C sources used in the experiment.

The C (C_{mic}) and N (N_{mic}) of the microbial biomass was analyzed using the fumigation-extraction method (Brookes *et al.*, 1985). Fresh soil subsamples (2.5 g) were fumigated with chloroform for 5 days. Non-fumigated subsamples served as controls. Then fumigated and non-fumigated samples were extracted with 12.5 ml of 0.5 M K_2SO_4 (in a ratio 1:5) by shaking for 1 h at 200 rpm at 20ºC in an orbital shaker and then filtering the suspension through a 0.45−μm Millipore filter (Jones & Willett, 2006; Delgado-Baquerizo *et al.*, 2015). The C_{mic} and N_{mic} concentrations were calculated as the difference between DOC or total N of fumigated and non-fumigated digested extracts, respectively. DOC was measured from these extracts by using a TOC analyzer (TOC–Vsch, Shimadzu, Kyoto, Japan). Total N was analyzed via a persulfate oxidation technique, whereas total N was oxidized to nitrate (D'Elia *et al.*, 1977) and then reduced to ammonium and analyzed using indophenol blue method in a microplate reader (Sims *et al.*, 1995). We used Kc=0.45 and Kn=0.54 as extractability correction factors (Brookes *et al.*, 1985; Joergensen, 1996).

Analyses of key components of the C and N cycle

We measured in all the soil samples different components of the C and N cycles. For the C cycle we measured total soil C, dissolved organic carbon (DOC) and main components of the dissolved organic matter such as hexoses, pentoses, amino acids and phenols. Total soil C was analyzed in soil samples with an elemental analyzer (THERMO SCIENTIFIC Mod. Flash 2000). To carry out the rest of the analyses, fresh soil subsamples (2.5 g) were extracted with 12.5 ml of 0.5 M K_2SO_4 (in a ratio 1:5) by shaking for 1 h at 200 rpm at 20ºC in an orbital shaker and then filtering the suspension through a 0.45-□m Millipore

filter (Jones & Willett, 2006; Delgado-Baquerizo *et al.*, 2015). DOC was measured from these extracts by using a TOC analyzer (TOC–Vsch, Shimadzu, Kyoto, Japan). The concentration of amino acids, hexoses, pentoses and phenols were assessed colorimetrically from the same extracts following Chantigny *et al.* (2008) by using the Ninhydrin (570 nm absorbance), the orcinol (655 nm absorbance), the anthrone (620 nm absorbance) and Folin-Ciocalteu (720 nm absorbance), respectively.

Regarding the N cycle, we measured dissolved organic nitrogen (DON), ammonium (NH_4^+-N), and nitrate (NO_3^--N). These measurements were determined colorimetrically from the soil extracts using a microplate reader as described by Durán *et al.* (2009) and Delgado-Baquerizo *et al.* (2011). To calculate DON concentration, we firstly oxidized the organic N dissolved in the extracts to NO_3^--N with potassium persulfate ($K_2S_2O_8$) in an autoclave at 121 ºC for 55 min, and then it was reduced with Devarda alloy to NH_4^+. Finally, the DON contents was calculated as the difference between the total N measured in the digested sample and the inorganic nitrogen (NH_4^+-N and NO_3^--N) of the sample (Robertson *et al.*, 1999). The NH_4^+-N concentration was determined directly using the indophenol blue method (Sims *et al.*, 1995). To determine NO_3^--N concentration, firstly NO_3^- in the extracts was reduced to NH_4^+ by incubating them in microplates with Devarda alloy overnight, then the NO_3^--N concentration was determined as the difference between the Devarda-incubated and incubated extracts.

Data analysis

Analyses of the microbial catabolic profile. Prior to the statistical analyses, we expressed the microbial catabolic profile in a format compatible with its inclusion as dependent variable in the neighborhood models. We used the results of the MicroResp™ analyses for the calculation of the Shannon-Weaver index, the Simpson-Yule index, and a dimensionality reduction using a Principal Components Analysis (PCA). The Shannon-Weaver index was calculated as $H' = \sum_{i=1}^{S} P_i \cdot \ln P_i$, where P_i is the ratio between the activity of a particular C source and the sum of activities of all C sources (Zak *et al.*, 1994). The Simpson-Yule index was calculated as $S = 1/\sum P_i$. The *H'* determine the capacity of the microbial community to catabolize a gradient of different C substrates, while *S* represents the catabolic evenness of respiration rates (McIntosh & Macdonald, 2013). Regarding the PCA, in both forest types the first component explained a large

proportion of the variation in the microbial catabolic profile (70% in woodlands and 73% in closed forests), thus it was used as a parsimonious summary of these data.

Neighborhood models of soil variables - For the analysis of our data we applied likelihood methods and model selection (Johnson & Omland, 2004; Canham & Uriarte, 2006; Gómez-Aparicio & Canham, 2008). We fit separated models for each combination of forest type (woodland and closed forest) and soil variables related to the microbial community (H', S, PC1, C_{mic}, N_{mic}, R_{mic}), the variables related to the C cycle (total soil C, DOC, hexoses, pentoses, amino acids, phenols) and the N cycle (NO_3^-, NH_4^+, DON). Our full model estimated two terms: 1) the average soil variable at each study site in the absence of specific effects of neighboring trees (a_{Site} in Eq 1 and 2), and 2) tree neighborhood effects on soil variables, which are assumed to vary as a function of a neighborhood index (NI). We included these neighborhood effects using both a linear (1) and an exponential (2) model framework (for a similar approach, see Baribault & Kobe, 2011; Ávila *et al.*, 2016):

Soil variable = $a_{Site} + b$ x NI (1)

Soil variable = a_{Site} x exp (b x NI) (2)

The parameter b defines the steepness of the variation in the soil variables due to an increment in NI. The NI quantifies the net effect of $j=1, \ldots, n$ neighboring trees of $i=1, \ldots, s$ species on the soil variable and it is related directly to the size (dbh) of the neighboring trees and inversely to the distance to neighbors (Boyden *et al.*, 2012; Ibáñez *et al.*, 2015):

$$NI = \sum_{i=0}^{s} \sum_{j=0}^{n} \lambda_i \frac{dbh_{ij}^{\alpha}}{dist_{ij}^{\beta}} \qquad (3)$$

where dbh_{ij} is the diameter at breast height of each of the jth neighboring tree of the ith species and $dist_{ij}$ is the distance of the jth neighboring tree of the ith species to the sampling point. The parameters α and β estimated by the model determine the shape of the effect of the tree size (α) and the distance to the neighbor (β) on the index. We introduced the species-specific parameter λ, that ranges between -1 and 1, to allow for differences among health status (i.e. healthy, defoliated and dead *Q. suber*) or species (*Q. suber*, *Q. canariensis*, *O. europaea*) in their effect on soil variables.

We tested three different candidate models of decreasing complexity to assess the effects of the tree community on the studied soil variables: 1) A model that explicitly

considered the effect of trees of different health status and species on soil properties ("Health + Tree species" model). This model calculated 4 different □values (healthy *Q. suber*, defoliated *Q. suber*, dead *Q. suber*, and the coexisting tree species□; 2) A model that ignored the health status of *Q. suber* and only considered differences among tree species on their impacts on soil variables ("Tree species" model). This model calculated 2 □values, one for *Q. suber* and one for the coexisting tree species (*O. europaea* in open woodlands and *Q. canariensis* in closed forests); and 3) A null model that ignored tree effects on soils ("No tree" model), and therefore calculated soil variables just as a function of the study site.

Parameter estimation and model selection - Soil variables were modeled with maximum likelihood using a simulated annealing algorithm (Goffe *et al.*, 1994). Model comparisons were conducted following information theory principles and the Akaike Information Criterion corrected for small sample size (AICc, Burnham & Anderson, 2002). Lower AIC_c values indicate best model performance. When the difference between a competitive model and the best model is lower than 2 units, models are considered to have the same empirical support. Asymptotic two-unit support intervals were calculated for each estimated parameter. The goodness-of-fit was determined with the R^2 of the regression between observed and predicted data. The slope of that regression was used to measure model bias, with unbiased prediction having a slope ~1. All statistical analyses were performed using R 3.3.2 (R Core Team, 2016) and the "likelihood" package (Murphy, 2012).

Results

Pathogen-induced tree mortality effects on the microbial community

We did not find any effect of tree species or health status on the microbial catabolic profile as indicated by the Shannon-Weaver index, the Simpson-Yale index, and the first component of the PCA. Thus, the simplest model (“No tree” model) that ignored the effects of trees on the microbial functional diversity offered a better fit to data than models that considered these effects in both forest types (Table 1). Our neighborhood models did not detect either any effect of trees on the C or N microbial biomass (Table 1). We however found an effect of the tree community on microbial respiration (i.e. 'Health + Tree species' or 'Tree species' models were selected as best models). Best respiration

models produced unbiased estimates of microbial respiration (i.e. slopes of predicted vs observed values were ~1.0) and R^2 ranged from 0.37 in closed forests to 0.50 in open woodlands (Table 1). Neighborhoods dominated by healthy trees of *Q. suber* and the coexistent species had higher microbial respiration than those dominated by defoliated or dead *Q. suber* trees. This effect was detected in both forests, although in closed forests a simpler model ignoring the health status of *Q. suber* had similar empirical support (i.e. ΔAIC<2 units) than a model including it. Among species, (healthy) *Q. suber* and coexistent tree species (*O. europaea* and *Q. canariensis*) had all similar positive impacts on microbial respiration, as indicated by overlapping positive λ values in both forest types (Tables S2 and S3).

Table 1 Comparison of alternative neighborhood models for microbial community variables (i.e. Shannon-Weaver index (H), Simpson-Yule index (S), first principal component (PC1), microbial respiration (R_{mic}), microbial C (C_{mic}) and microbial N (N_{mic})), in the two forest types (open woodlands and closed forests) using the Akaike Information Criterion corrected for small sample sizes (AICc). ΔAICc represents the differences between the AICc of the model and the AICc of the best model (i.e. the model with the lowest AICc, indicated in bold). 'Health + Tree species' models include differences among tree species and health status in their effects on soils, 'Tree species' models consider only differences among tree species, and 'No tree' models do not consider any tree effects. 'Mod' indicates a linear (Lin) or an exponential (Exp) relationship between soil variables and tree effects. 'Par' is the total number of parameters in the best model. The slope ('SL') and R^2 for the relationship between predicted and observed values are also given when tree effects were detected.

		ΔAICc						
Type of forest	Soil variable	Health + Tree species	Tree species	No tree	Mod	Par	SL	R^2
Open woodlands	H	15.30	10.67	**0.00**	-	4	-	-
	S	13.86	8.92	**0.00**	-	4	-	-
	PC1	10.97	9.61	**0.00**	-	4	-	-
	R_{mic}	**0.00**	6.28	2.34	Exp	11	1.00	0.50
	C_{mic}	5.80	8.47	**0.00**	-	2	-	-
	N_{mic}	8.85	7.62	**0.00**	-	2	-	-
Closed forests	H	14.25	9.88	**0.00**	-	2	-	-
	S	10.19	8.35	**0.00**	-	2	-	-
	PC1	6.85	3.57	**0.00**	-	4	-	-
	R_{mic}	1.11	**0.00**	10.93	Exp	9	1.00	0.37
	C_{mic}	8.31	5.88	**0.00**	-	2	-	-
	N_{mic}	8.89	8.48	**0.00**	-	2	-	-

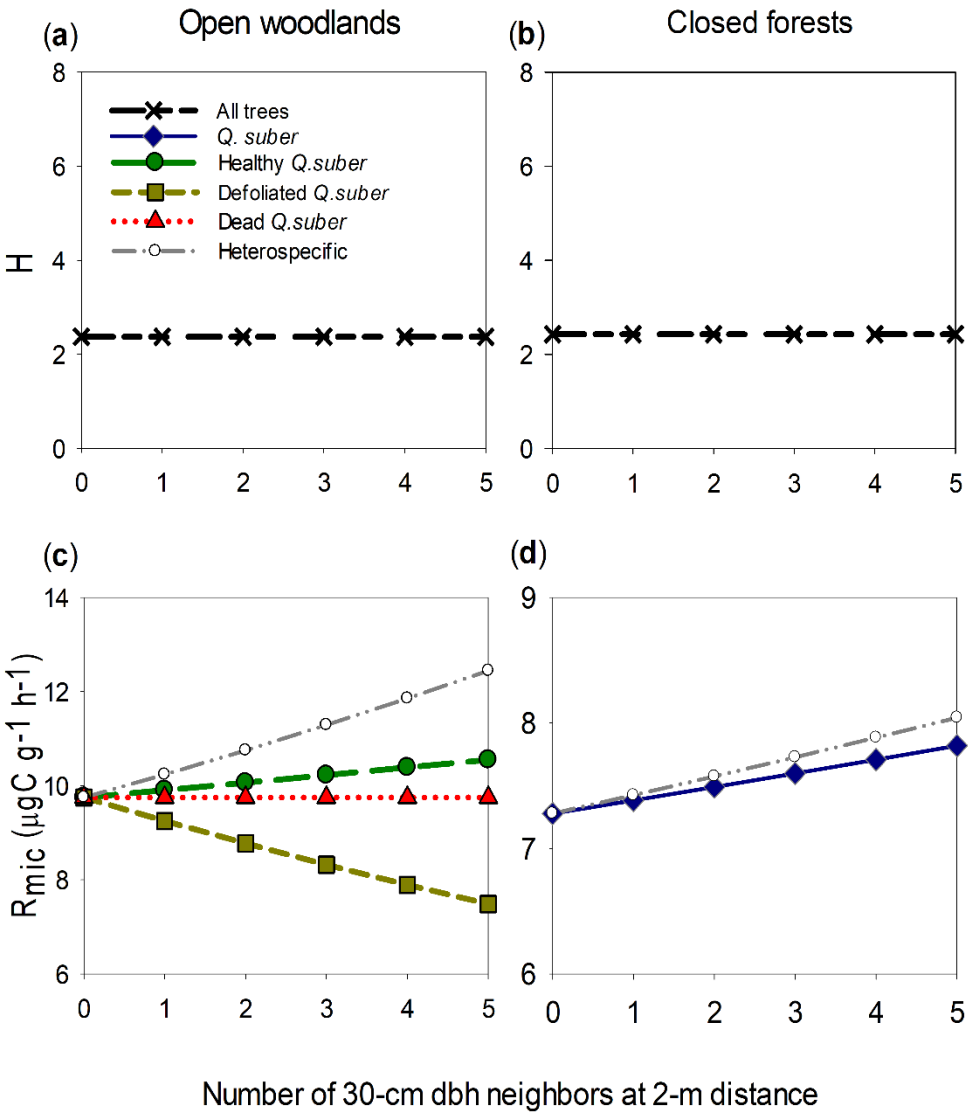


Figure 1 Predicted effects of variation in neighbour identity and quantity in open woodlands and closed forests on microbial community variables: (a, b) H Shannon-Weaver index, (c, d) microbial respiration (R_{mic}). Predictions were done using parameter values from best models (Supporting Information Tables S2, S3). The number (0-5) of average-sized neighbours (i.e. d.b.h. = 30 cm) at 2-m distance represent the range of neighbour abundance found in our forests for that distance.

Pathogen-induced tree mortality effects on the soil organic matter and N pools

All best models produced unbiased estimates of C and N-related variables and R^2 ranged from 0.10 to 0.50 (Table 2). We detected an effect of the tree community on C-related variables, but these effects varied between forest types. In open woodlands, the most complex model considering health and tree species effects (i.e. "Health + Tree species" model) was the best fit for total soil C and hexoses, whereas no tree effects were detected for the four remaining C-related variables (i.e. the "No tree" model offered the best fit,

Table 2). In this forest type, neighborhoods dominated by healthy *Q. suber* trees had lower total soil C than neighborhoods dominated by defoliated and dead trees, while neighborhoods dominated by healthy and dead trees had higher hexoses than neighborhoods dominated by defoliated trees (Fig 2). In closed forests, the "Health + Tree species" model offered the best fit for four of the six C-related variables (i.e. total soil C, DOC, hexoses and pentoses). Neighborhoods dominated by healthy and defoliated *Q. suber* trees had higher total soil C but lower DOC than those dominated by dead trees (Fig 2, see λ values in Tables S2 and S3). Neighborhoods dominated by healthy *Q. suber* trees in closed forests also had higher pentoses but lower hexoses than those dominated by defoliated and dead trees. Moreover, our models detected a negative effect of trees (i.e. *Q. suber* and *Q. canariensis*) on phenols in closed forests, whereas no tree effects were detected for amino acids (Table 2, Fig 2). Among species, *Q. suber* and *O. europaea* had similar impacts on all the studied C-related variables in open woodlands (i.e. overlapping λ values, Table S2). In closed forests, on the contrary, *Q. suber* and *Q. canariensis* showed differential impacts on DOC and hexoses. DOC was lower and hexoses were higher in soils under *Q. suber* than in soils under *Q. canariensis* trees (Fig 2, Table S3).

We did not detect any effect of tree species or health status on DON (i.e. the simplest "No tree" model was the fit in both forest types). Our neighborhood models detected effects of the health status of *Q. suber* trees on soil N availability (NH_4^+ and NO_3^-), but only in open woodlands (Table 2). In this forest type, neighborhoods dominated by healthy *Q. suber* trees had generally higher levels of NH_4^+ and lower levels of NO_3^- than neighborhoods dominated by dead *Q. suber* trees (Fig 3). In both forest types, best models for NH_4^+ and NO_3^- also considered tree species effects (Table 2). Among species, soils under (healthy) *Q. suber* trees had higher NH_4^+ but similar NO_3^- than *O. europaea* trees. Moreover, we detected lower NH_4^+ but higher NO_3^- in soils under *Q. suber* trees than under *Q. canariensis* trees (Fig 3).

Table 2 Comparison of alternative neighborhood models for C and N variables (i.e. total soil C, dissolved organic carbon (DOC), hexoses, pentoses, amino acids, phenols, dissolved organic nitrogen (DON), ammonium (NH_4^+) and nitrate (NO_3^-)), in the two forest types (woodlands and closed forests) using the Akaike Information Criterion corrected for small sample sizes (AICc). ΔAICc represents the differences between the AICc of the model and the AICc of the best model (i.e. the model with the lowest AICc, indicated in bold). 'Health + Tree species' models include differences among tree species and health status in their effects on soils, 'Tree species' models consider only differences among tree species, and 'No tree' models do not consider any tree effects. 'Mod' indicates a linear (Lin) or an exponential (Exp) relationship between soil variables and tree effects. 'Par' is the total number of parameters in the best model. The slope ('SL') and R^2 for the relationship between predicted and observed values are also given when tree effects were detected.

		ΔAICc						
Type of forest	Soil variable	Health + Tree species	Tree species	No tree	Mod	Par	SL	R^2
Open woodlands	Soil C	**0.00**	1.89	4.57	Lin	11	1.00	0.17
	DOC	10.40	1.72	**0.00**	-	4	-	-
	Hexoses	**0.00**	7.42	18.30	Lin	11	0.95	0.23
	Pentoses	6.28	5.54	**0.00**	-	4	-	-
	Amino acids	5.09	6.78	**0.00**	-	4	-	-
	Phenols	8.42	12.42	**0.00**	-	4	-	-
	DON	11.29	7.34	**0.00**	-	4	-	-
	NH_4^+	**0.00**	1.02	49.47	Lin	11	1.04	0.28
	NO_3^-	**0.00**	10.76	1.71	Lin	11	1.04	0.13
Closed forests	Soil C	**0.00**	3.84	16.78	Lin	11	1.00	0.10
	DOC	**0.00**	10.88	41.31	Lin	11	0.97	0.26
	Hexoses	**0.00**	3.32	2.69	Lin	11	0.97	0.15
	Pentoses	**0.00**	9.81	22.07	Lin	11	1.05	0.14
	Amino acids	9.50	9.19	**0.00**	-	4	-	-
	Phenols	4.85	**0.00**	9.15	Exp	9	0.99	0.10
	DON	8.17	3.70	**0.00**	-	4	-	-
	NH_4^+	4.82	**0.00**	17.94	Exp	9	0.99	0.11
	NO_3^-	8.28	**0.00**	24.95	Exp	9	1.05	0.10

Figure 2 Predicted effects of variation in neighbour identity and quantity in open woodlands (a-f) and closed forests (g-l) on C-related variables: Soil C, DOC (dissolved organic carbon), hexoses, pentoses, aminoacids and phenols. Predictions were done using parameter values from best models (Supporting Information Tables S2, S3). The number (0-5) of average-sized neighbours (i.e. d.b.h. = 30 cm) at 2-m distance represent the range of neighbour abundance found in our forests for that distance.

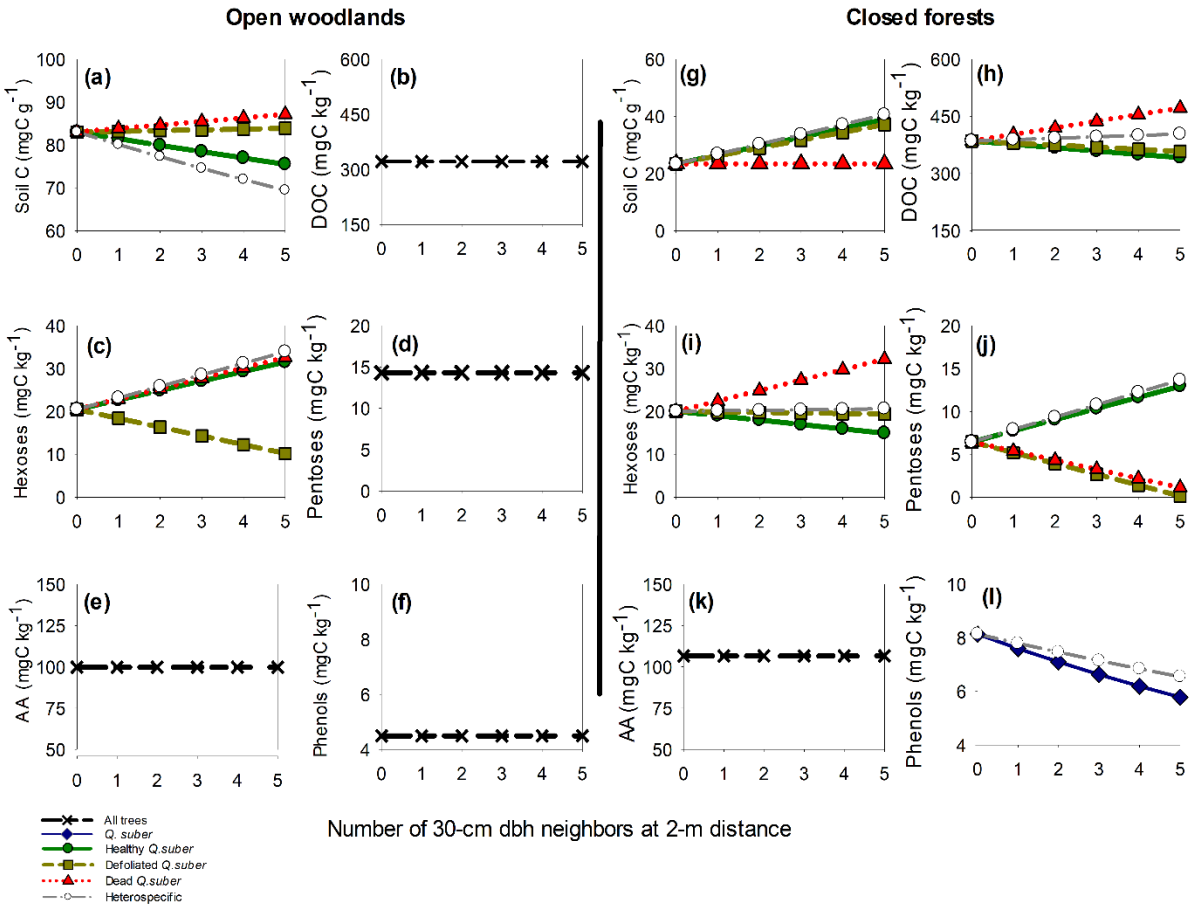
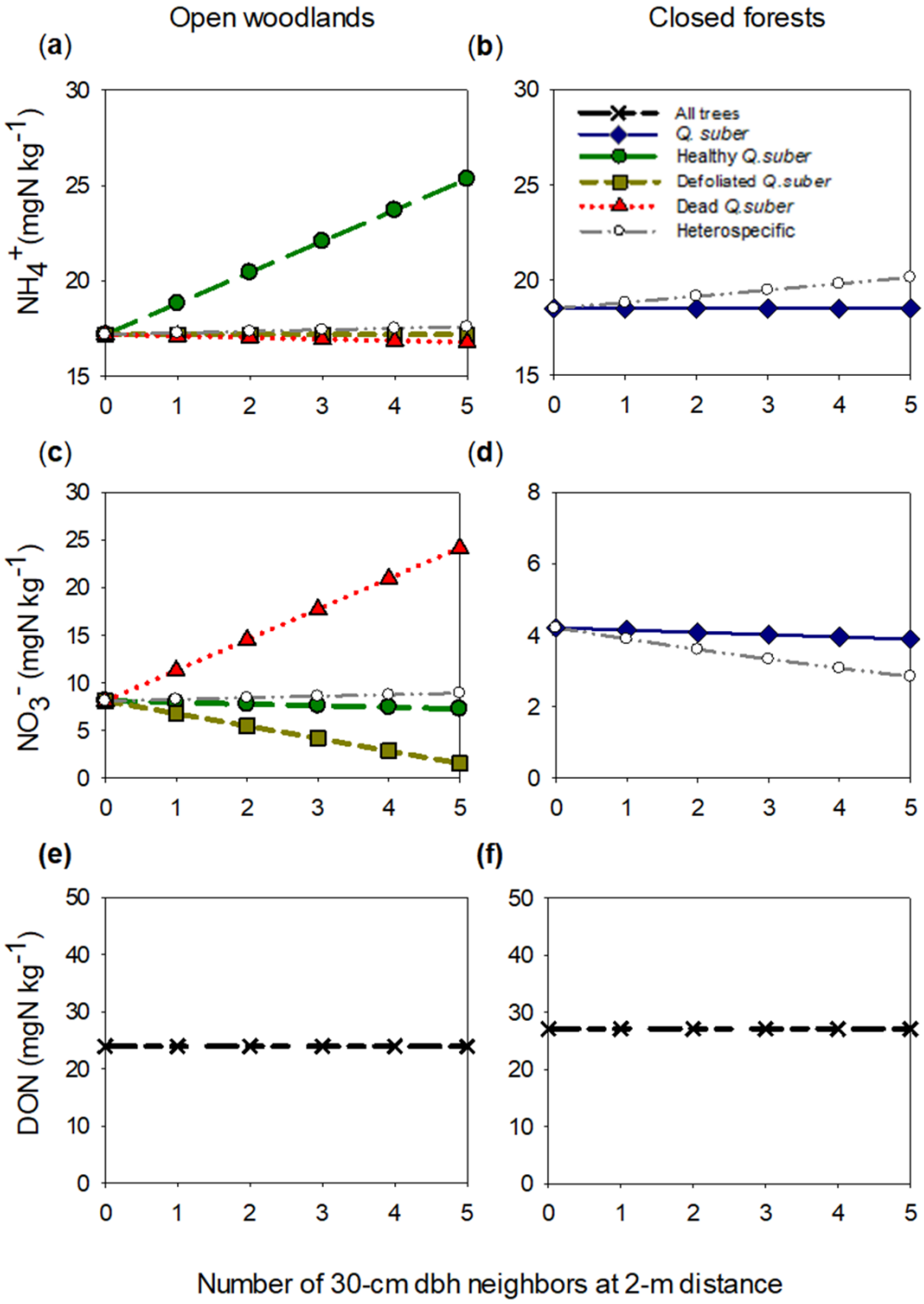


Figure 3 Predicted effects of variation in neighbour identity and quantity in open woodlands and closed forests on inorganic and organic N: (a, b) DON (dissolved organic nitrogen), (c, d) NH_4^+ and (e, f) NO_3^-. Predictions were done using parameter values from best models (Supporting Information Tables S2, S3). The number (0-5) of average-sized neighbours (i.e. d.b.h. = 30 cm) at 2-m distance represent the range of neighbour abundance found in our forests for that distance.



Discussion

This study provides compelling evidence showing that the decline of a dominant tree species (i.e. *Q. suber*) induced by an exotic soil-borne pathogen (i.e. *P. cinnamomi*) in Mediterranean forests translated into substantial modifications of the C and N cycle, but with no effects on the microbial community in terms of functional diversity or microbial biomass. The spatially-explicit neighborhood approach applied in this study at a large spatial scale (2 forest types, 6 sites) allowed providing valuable insights that contribute to increase our knowledge about the consequences of tree mortality driven by biotic agents on soil biology.

Direct effects of pathogen-induced tree mortality on the microbial community

Soil microorganisms are involved in numerous key processes such as soil formation, organic matter decomposition or nutrient cycling (e.g. Zak *et al.*, 2003; Van Der Heijden *et al.*, 2008; Bardgett & Wardle, 2010). Therefore, the study of the functional diversity, biomass and functioning of microbial communities is of crucial importance to understand changes in soil functioning after biotic disturbances (Chodak *et al.*, 2016). Our models did not detect any effect of the pathogen-induced tree mortality on microbial functional diversity, contrary to our first hypothesis. Previous studies on tree decline had found a decrease in microbial functional diversity with declining tree health, likely as a result of changes in the quality and quantity of leaf litter and/or root exudates that imply a reduced number of C sources under declining trees (Cai *et al.*, 2010; Rodríguez *et al.*, 2016). In our study, however, the absence of direct effects of *Q. suber* decline on the functional diversity of microbial communities implies that the microbial community under healthy and affected trees was able to use the same substrates, therefore suggesting that the relative composition of C substrates was not substantially altered by the process of decline.

We expected an increase in microbial biomass and respiration under declining *Q. suber* trees as a consequence of the increase in C inputs and N availability frequently associated to biotic disturbances (Nave *et al.*, 2011; Edburg *et al.*, 2012; Zhang *et al.*, 2015). However, and again contrary to our first hypothesis, we found no direct effects of pathogen-driven tree decline on microbial biomass and a decrease in microbial respiration. In a previous field study conducted in the same study sites, a decrease in soil respiration associated to the process of *Q. suber* defoliation and death was also found

(Ávila *et al.*, 2016). This result was interpreted as a reduction of the tree C allocation belowground, and consequently of root and rhizosphere respiration (i.e. autotrophic respiration). However, results from this study indicate that not only autotrophic respiration is reduced in the context of pathogen-induced mortality, but also heterotrophic microbial respiration. Moreover, our findings support the idea that the positive relationship between soil biodiversity and ecosystem functioning is no universally found (e.g. Bell *et al.*, 2009), and that global change drivers can result in substantial changes in ecosystem functioning even with no alteration of the abundance and functional diversity of the microbial community.

Direct effects of pathogen-induced tree mortality on soil organic matter

Our results showed that pathogen-induced *Q. suber* mortality resulted in important changes of key components of the C cycle in Mediterranean forests. However, these changes were not consistent among forest types. We predicted a reduction in C pools with high residence times (e.g. total soil C, phenols) and an increase in C sources with low residence times (e.g. DOC, hexoses) as a consequence of pathogen-induced tree mortality (our second hypothesis). However, this hypothesis was supported just in closed forests, where our models predicted a strong reduction (up to 40%) of total soil C in neighborhoods dominated by dead *Q. suber* trees compared to healthy neighborhoods (Fig. 2g), but an increase in DOC and particularly in hexoses (two-fold increase, Fig 2i). Previous reviews have shown that during the first years of the tree decline process, a decrease in soil total C can occur due to reductions in net primary productivity and an increase in decomposition rates (Hicke *et al.*, 2012; Zhang *et al.*, 2015). Despite this reduction in total soil C, an increase in the more labile forms of C under defoliated and dead trees might be also expected due to decomposition of the large inputs of litterfall and root biomass released during the decline process (Zhang *et al.*, 2015). The variation in hexoses and pentoses found in closed forests support these ideas. Thus, our models showed a reduction in pentoses (considered plant-derived carbohydrates; (Chesire, 1979; Chantigny *et al.*, 2008) but an increase of hexoses (considered microbial-derived carbohydrates) under dead trees. These changes denoted an alteration of the carbohydrate origin in the soils following pathogen-induced mortality, moving from plant-derived carbohydrates to microbial-derived carbohydrates. This suggests a decrease in the role of plant compounds in the decomposition process under dead *Q. suber* trees, probably due to reductions in primary productivity.

Interestingly, we found a contrasted pattern in open woodlands where we detected an increase in total soil C under defoliated and dead trees in comparison with healthy trees. A plausible explanation for the contrasting results found among forest types could lie in their differential texture characteristics. In open woodlands, the high clay content might protect the organic matter against decomposition, since clay plays a well-known key role in the physical protection of soil organic matter (e.g. Van Veen & Kuikman, 1990; Hassink & Whitmore, 1997; Müller & Höper, 2004). This hypothesis was supported by our data, since soils in open woodlands had higher total soil C but lower DOC than closed forests, which could be interpreted as an accumulation of soil organic matter in such soils. Moreover, if we compared the metabolic quotient (qCO_2, i.e. respiration per unit of microbial biomass) among forest types, we found lower qCO_2 in open woodlands than in closed forests, indicating that soil microorganisms in open woodlands were less efficient in their carbon use, probably due to the protective role of clay (Franzluebbers *et al.*, 1996; Müller & Höper, 2004).

Overall, our results clearly showed the large capacity of pathogen-induced tree mortality to modify key soils variables of relevance for the C cycle in Mediterranean forests, but also demonstrated that the exact sign and magnitude of such effects can be largely context-dependent, as we predicted in the fourth hypothesis. Information on local soil characteristics such as texture seem to be essential to accurately predict the impact of biotic disturbances on the dynamics of soil organic matter, with potential different outcomes depending on whether soil organic matter is controlled just by microbial activity (sandy soils) or subjected also to a mineralogical control (clay soils).

Direct effects of pathogen-induced tree mortality on soil N pools

Our results showed that the defoliation and death of *Q. suber* translated into substantial alterations in soil inorganic N, but not in soil organic N. Although some studies have assessed the effect of biotic disturbances on inorganic N, very few studies have evaluated their consequences on DON despite its importance on soil processes such as depolymerization and N mineralization (Schimel & Bennett, 2004). To our knowledge, the only published work on the effect of biotic disturbances on DON in forests found higher values of this variable in stands of *Tsuga canadensis* affected by the insect *Adelgies tsugae* (hemlock woolly adelgid) than in non-affected stands due to the accumulation of frass, insect biomass and senescent leaf tissue under affected trees (Stadler *et al.*, 2005). It could be possible that biotic disturbances involving insects have

a larger capacity to rapidly alter soil DON than disturbances involving pathogens. Clearly, further studies are needed before any robust conclusions can be reached regarding the potential of biotically-driven tree mortality to modify soil DON levels.

Regarding inorganic N, we expected an increase in N concentration under defoliated and dead trees (third hypothesis). Our findings partially agree with this hypothesis, since we found an increase in NO_3^- but a decrease in NH_4^+ under dead trees, probably due to an increment in nitrification rates. However, this effect was only detected in open woodlands, probably also influenced by the effect of texture. In soils with higher clay content (such as those in woodlands) inorganic N can be retained in clay particles (Allison, 1973; Hassink, 1997), whereas in sandy soils (such as those in closed forests) the high diffusion of inorganic N could eliminate differences among healthy and declining trees in soil N concentration (Vitousek & Melillo, 1979; Weil *et al.*, 2016). Overall, our results indicate that pathogen-induced oak mortality might lead to significant changes in the concentration of soil inorganic N with no impacts on organic N, and that such effects on the N cycle might be controlled by soil texture as found for the C cycle.

Indirect effects of oak decline on the C and N cycle

In the studied mixed forests, *P. cinnamomi*-induced mortality severely affects *Q. suber* trees with no apparent effects on coexistent tree species, i.e. *O. europaea* in open woodlands and *Q. canariensis* in closed forests. This species-specific disturbance might induce in the long term a species replacement that could lead to indirect impacts on ecosystem processes, as it has been documented in previous works for other biotic disturbances (e.g. Lovett *et al.*, 2010; Cobb *et al.*, 2013). We found differences among species in their effects on soil variables that might translate into altered ecosystem functioning if such substitution of species occurs. For instance, we found lower inorganic N under *O. europaea* than under *Q. suber*, while the footprint of *Q. canariensis* on soil C and N pools was characterized by higher DOC, hexoses and NH_4^+ than the footprint of *Q. suber*. Therefore, a potential substitution of *Q. suber* by *O. europaea* might result in soils impoverished in N, whereas a replacement by *Q. canariensis* could lead to more fertile soils with higher content of soil organic C and inorganic N. These results clearly highlight the importance of the identity of the replacement species when predicting the potential long-term effects of selective biotic disturbances in forests.

Concluding remarks

With this work we provide valuable insights to advance our understanding of the consequences of global change-related biotic disturbance such as invasive pathogens on belowground processes. Our results showed complex short- and long-term effects of pathogen-induced tree mortality on different components of the C and N cycle, with no effects on the functional diversity or the biomass of the microbial community. Although it has been shown that losses of soil diversity due to global change can translate into reductions in soil functioning (García-Palacios *et al.*, 2013; Delgado-Baquerizo *et al.*, 2015), this study showed that negative effects on soil functioning can be detected even independently of changes in soil (functional) diversity. Moreover, we found that the effect of pathogen-induced mortality on C and N differed in sign and magnitude between forests with contrasted soil characteristics, particularly texture. It has been recently recognized that differences in soil texture might lead to divergent responses of forests systems to global change (Ibáñez *et al.*, 2014; Case & Staver, 2018). Our study adds to this line of evidence suggesting a key role of texture as a modifier of the impacts of pathogen-induced tree mortality on C and N cycles. The findings of this study are of special relevance in the current context of increasing influx and spread of invasive forests pests and diseases, which represent a severe risk to forest functioning and their associated ecosystem services worldwide.

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References

Allen, C.D., Breshears, D.D., McDowell, N.G., 2015. On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. Ecosphere 6, art129.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.H., Allard, G., Running, S.W., Semerci, A., Cobb, N., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management 259, 660-684.

Allison, F.E., 1973. Nonbiological Immobilization of Nitrogen, Developments in Soil Science, Volume 3. Soil Organic Matter and its Role in Crop Production. Elsevier, Amsterdam, pp. 206-229.

Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences 105, 11512-11519.

Aponte, C., García, L.V., Pérez-Ramos, I.M., Gutiérrez, E., Marañón, T., 2011. Oak trees and soil interactions in Mediterranean forests: A positive feedback model. Journal of Vegetation Science 22, 856-867.

Ávila, J.M., Gallardo, A., Ibáñez, B., Gómez-Aparicio, L., 2016. Quercus suber dieback alters soil respiration and nutrient availability in Mediterranean forests. Journal of Ecology 104, 1441-1452.

Ávila, J.M., Linares, J.C., García-Nogales, A., Sánchez, M.E., Gómez-Aparicio, L., 2017. Across-scale patterning of plant–soil–pathogen interactions in Quercus suber decline. European Journal of Forest Research 136, 677-688.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505.

Bardgett, R.D., Wardle, D.A., 2010. Aboveground-Belowground Linkages: Biotic Interactions, Ecosystem Processes, and Global Change. Oxford University Press, New York, USA.

Baribault, T.W., Kobe, R.K., 2011. Neighbour interactions strengthen with increased soil resources in a northern hardwood forest. Journal of Ecology 99, 1358-1372.

Bell, T., Gessner, M.O., Griffiths, R.I., McLaren, J.R., Morin, P.J., van der Heijden, M., van der Putten, W., 2009. Microbial biodiversity and ecosystem functioning under controlled conditions and in the wild. Biodiversity, Ecosystem Functioning, and Human Wellbeing: An Ecological and Economic Perspective. Oxford University Press: Oxford, 121-133.

Boyden, S., Montgomery, R., Reich, P.B., Palik, B., 2012. Seeing the forest for the heterogeneous trees: stand-scale resource distributions emerge from tree-scale structure. Ecological Applications 22, 1578-1588.

Brasier, C.M., 1992. Oak tree mortality in Iberia. Nature 360, 539.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17, 837-842.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multi-Model Inference : A Practical Information-Theoretic Approach, Second edition ed. Springer, Secaucus, NJ, USA.

Cai, Y.F., Barber, P., Dell, B., O'Brien, P., Williams, N., Bowen, B., Hardy, G., 2010. Soil bacterial functional diversity is associated with the decline of Eucalyptus gomphocephala. Forest Ecology and Management 260, 1047-1057.

Camilo-Alves, C.S.P., da Clara, M.I.E., Ribeiro, N.A., 2013. Decline of Mediterranean oak trees and its association with Phytophthora cinnamomi: a review. European Journal of Forest Research 132, 411-432.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Applied and Environmental Microbiology 69, 3593-3599.

Canham, C.D., Uriarte, M., 2006. Analysis of neighborhood dynamics of forest ecosystems using likelihood methods and modeling. Ecological Applications 16, 62-73.

Case, M.F., Staver, A.C., 2018. Soil texture mediates tree responses to rainfall intensity in African savannas. New Phytologist 219, 1363-1372.

Cobb, R.C., Eviner, V.T., Rizzo, D.M., 2013. Mortality and community changes drive sudden oak death impacts on litterfall and soil nitrogen cycling. New Phytologist 200, 422-431.

Chantigny, M.H., Angers, D.A., Kaiser, K., Kalbitz, K., 2008. Extraction and characterization of dissolved organic matter, In: Carter, M.R., Gregorich, E.G. (Eds.), Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, pp. 617–635.

Chesire, M.V., 1979. Nature and Origin of Carbohydrates in Soils. Academic Press, London.

Chodak, M., Klimek, B., Niklińska, M., 2016. Composition and activity of soil microbial communities in different types of temperate forests. Biology and Fertility of Soils 52, 1093-1104.

D'Elia, C.F., Steudler, P.A., Corwin, N., 1977. Determination of Total Nitrogen in Aqueous Samples Using Persulfate Digestion. Limnology and Oceanography 22, 760-764.

Delgado-Baquerizo, M., Covelo, F., Gallardo, A., 2011. Dissolved Organic Nitrogen in Mediterranean Ecosystems. Pedosphere 21, 309-318.

Delgado-Baquerizo, M., García-Palacios, P., Milla, R., Gallardo, A., Maestre, F.T., 2015. Soil characteristics determine soil carbon and nitrogen availability during leaf litter decomposition regardless of litter quality. Soil Biology and Biochemistry 81, 134-142.

Delgado-Baquerizo, M., Giaramida, L., Reich, P.B., Khachane, A.N., Hamonts, K., Edwards, C., Lawton, L.A., Singh, B.K., Brophy, C., 2016a. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. Journal of Ecology 104, 936-946.

Delgado-Baquerizo, M., Grinyer, J., Reich, P.B., Singh, B.K., Allen, E., 2016b. Relative importance of soil properties and microbial community for soil functionality: insights from a microbial swap experiment. Functional Ecology 30, 1862-1873.

Delgado-Baquerizo, M., Morillas, L., Maestre, F.T., Gallardo, A., 2013. Biocrusts control the nitrogen dynamics and microbial functional diversity of semi-arid soils in response to nutrient additions. Plant and Soil 372, 643-654.

Durán, J., Rodríguez, A., Fernández-Palacios, J.M., Gallardo, A., 2009. Changes in net N mineralization rates and soil N and P pools in a pine forest wildfire chronosequence. Biology and Fertility of Soils 45, 781-788.

Edburg, S.L., Hicke, J.A., Brooks, P.D., Pendall, E.G., Ewers, B.E., Norton, U., Gochis, D., Gutmann, E.D., Meddens, A.J.H., 2012. Cascading impacts of bark beetle-

caused tree mortality on coupled biogeophysical and biogeochemical processes. Frontiers in Ecology and the Environment 10, 416-424.

Franzluebbers, A.J., Haney, R.L., Hons, F.M., Zuberer, D.A., 1996. Active fractions of organic matter in soils with different texture. Soil Biology and Biochemistry 28, 1367-1372.

García-Palacios, P., Bowker, M.A., Chapman, S.J., Maestre, F.T., Soliveres, S., Gallardo, A., Valladares, F., Guerrero, C., Escudero, A., 2011. Early-successional vegetation changes after roadside prairie restoration modify processes related with soil functioning by changing microbial functional diversity. Soil Biology and Biochemistry 43, 1245-1253.

García-Palacios, P., Milla, R., Delgado-Baquerizo, M., Martín-Robles, N., Álvaro-Sánchez, M., Wall, D.H., 2013. Side-effects of plant domestication: ecosystem impacts of changes in litter quality. New Phytologist 198, 504-513.

Goffe, W.L., Ferrier, G.D., Rogers, J., 1994. Global optimization of statistical functions with simulated annealing. Journal of Econometrics 60, 65-99.

Gómez-Aparicio, L., Canham, C.D., 2008. Neighborhood models of the effects of invasive tree species on ecosystem processes. Ecological Monographs 78, 69-86.

Gómez-Aparicio, L., Ibáñez, B., Serrano, M.S., De Vita, P., Ávila, J.M., Pérez-Ramos, I.M., García, L.V., Sánchez, M.E., Marañón, T., 2012. Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration. New Phytologist 194, 1014-1024.

Hancock, J.E., Arthur, M.A., Weathers, K.C., Lovett, G.M., 2008. Carbon cycling along a gradient of beech bark disease impact in the Catskill Mountains, New York. Canadian Journal of Forest Research 38, 1267-1274.

Hassink, J., 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant and Soil 191, 77-87.

Hassink, J., Whitmore, A.P., 1997. A model of the physical protection of organic matter in soils. Soil Science Society of America Journal 61, 131-139.

Hättenschwiler, S., Tiunov, A.V., Scheu, S., 2005. Biodiversity and Litter Decomposition in Terrestrial Ecosystems. Annual Review of Ecology, Evolution, and Systematics 36, 191-218.

Hicke, J.A., Allen, C.D., Desai, A.R., Dietze, M.C., Hall, R.J., Hogg, E.H., Kashian, D.M., Moore, D., Raffa, K.F., Sturrock, R.N., Vogelmann, J., 2012. Effects of

biotic disturbances on forest carbon cycling in the United States and Canada. Global Change Biology 18, 7-34.

Hobara, S., Tokuchi, N., Ohte, N., Koba, K., Katsuyama, M., Kim, S.J., Nakanishi, A., 2001. Mechanism of nitrate loss from a forested catchment following a small-scale, natural disturbance. Canadian Journal of Forest Research 31, 1326-1335.

Holden, S.R., Treseder, K.K., 2013. A meta-analysis of soil microbial biomass responses to forest disturbances. Frontiers in Microbiology 4.

Ibáñez, B., Gómez-Aparicio, L., Stoll, P., Ávila, J.M., Pérez-Ramos, I.M., Marañón, T., 2015. A neighborhood analysis of the consequences of Quercus suber decline for regeneration dynamics in Mediterranean forests. PloS one 10, e0117827.

Ibáñez, B., Ibáñez, I., Gómez-Aparicio, L., Ruiz-Benito, P., García, L.V., Marañón, T., 2014. Contrasting effects of climate change along life stages of a dominant tree species: the importance of soil–climate interactions. Diversity and Distributions, n/a-n/a.

Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the kEC value. Soil Biology and Biochemistry 28, 25-31.

Johnson, J.B., Omland, K.S., 2004. Model selection in ecology and evolution. Trends in Ecology and Evolution 19, 101-108.

Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology and Biochemistry 38, 991-999.

Lovett, G.M., Arthur, M.A., Weathers, K.C., Griffin, J.M., 2010. Long-term changes in forest carbon and nitrogen cycling caused by an introduced pest/pathogen complex. Ecosystems 13, 1188-1200.

Lovett, G.M., Weiss, M., Liebhold, A.M., Holmes, T.P., Leung, B., Lambert, K.F., Orwig, D.A., Campbell, F.T., Rosenthal, J., McCullough, D.G., 2016. Nonnative forest insects and pathogens in the United States: Impacts and policy options. Ecological Applications 26, 1437-1455.

Lowe, S., Browne, M., Boudjelas, S., De Poorter, M., 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group Species Survival Commission, World Conservation Union (IUCN), Auckland, New Zealand.

McIntosh, A.C.S., Macdonald, S.E., 2013. Short-term resistance of ecosystem properties and processes to simulated mountain pine beetle attack in a novel region. Ecosphere 4, art68.

Morehouse, K., Johns, T., Kaye, J., Kaye, A., 2008. Carbon and nitrogen cycling immediately following bark beetle outbreaks in southwestern ponderosa pine forests. Forest Ecology and Management 255, 2698-2708.

Müller, T., Höper, H., 2004. Soil organic matter turnover as a function of the soil clay content: consequences for model applications. Soil Biology and Biochemistry 36, 877-888.

Murphy, L., 2012. Likelihood: Methods for maximum likelihood estimation. R package version 1.6., 1.6 ed.

Nave, L.E., Gough, C.M., Maurer, K.D., Bohrer, G., Hardiman, B.S., Le Moine, J., Munoz, A.B., Nadelhoffer, K.J., Sparks, J.P., Strahm, B.D., Vogel, C.S., Curtis, P.S., 2011. Disturbance and the resilience of coupled carbon and nitrogen cycling in a north temperate forest. Journal of Geophysical Research-Biogeosciences 116, G04016.

Ninyerola, M., Pons, X., Roure, J.M., 2005. Atlas climático digital de la Península Ibérica: metodología y aplicaciones en bioclimatología y geobotánica. Universidad Autónoma de Barcelona, Barcelona.

Ojeda, F., Marañón, T., Arroyo, J., 2000. Plant diversity patterns in the Aljibe Mountains (S. Spain): A comprehensive account. Biodiversity and Conservation 9, 1323-1343.

Oren, A., Steinberger, Y., 2008. Catabolic profiles of soil fungal communities along a geographic climatic gradient in Israel. Soil Biology and Biochemistry 40, 2578-2587.

R Core Team, 2016. R: A language and environment for statistical computing, 3.3.2 ed. R Foundation for Statistical Computing, Vienna, Austria.

Robertson, G.P., Wedin, D., Groffmann, P., Blair, J., Holland, E., Nadelhoffer, K., Harris, D., 1999. Soil carbon and nitrogen availability: nitrogen mineralization, nitrification, and soil respiration potentials, Standard soil methods for long-term ecological research. Oxford University Press, pp. 258-271.

Rodríguez, A., Curiel Yuste, J., Rey, A., Durán, J., García-Camacho, R., Gallardo, A., Valladares, F., 2016. Holm oak decline triggers changes in plant succession and

microbial communities, with implications for ecosystem C and N cycling. Plant and Soil, 1-17.

Sánchez, M.E., Caetano, P., Ferraz, J., Trapero, A., 2002. Phytophthora disease of Quercus ilex in south-western Spain. Forest Pathology 32, 5-18.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: Challenges of a changing paradigm. Ecology 85, 591-602.

Schlesinger, W.H., Bernhardt, E.S., 2012. Biogeochemistry: An Analysis of Global Change. Elsevier Science.

Sims, G.K., Ellsworth, T.R., Mulvaney, R.L., 1995. Microscale determination of inorganic nitrogen in water and soil extracts. Communications in Soil Science and Plant Analysis 26, 303-316.

Stadler, B., Müller, T., Orwig, D., Cobb, R., 2005. Hemlock woolly adelgid in New England forests: Canopy impacts transforming ecosystem processes and landscapes. Ecosystems 8, 233-247.

Trumbore, S., Brando, P., Hartmann, H., 2015. Forest health and global change. Science 349, 814-818.

Urbieta, I.R., Zavala, M.A., Marañón, T., 2008. Human and non-human determinants of forest composition in southern Spain: Evidence of shifts towards cork oak dominance as a result of management over the past century. Journal of Biogeography 35, 1688-1700.

Van Der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11, 651.

Van Der Putten, W.H., Bardgett, R.D., De Ruiter, P.C., Hol, W.H.G., Meyer, K.M., Bezemer, T.M., Bradford, M.A., Christensen, S., Eppinga, M.B., Fukami, T., Hemerik, L., Molofsky, J., Schädler, M., Scherber, C., Strauss, S.Y., Vos, M., Wardle, D.A., 2009. Empirical and theoretical challenges in aboveground-belowground ecology. Oecologia 161, 1-14.

Van Veen, J.A., Kuikman, P.J., 1990. Soil structural aspects of decomposition of organic matter by micro-organisms. Biogeochemistry 11, 213-233.

Vitousek, P.M., Melillo, J.M., 1979. Nitrate losses from disturbed forests: patterns and mechanisms. Forest Science 25, 605-619.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Putten, W.H.v.d., Wall, D.H., 2004. Ecological Linkages between Aboveground and Belowground Biota. Science 304, 1629-1633.

Wardle, D.A., Peltzer, D.A., 2017. Impacts of invasive biota in forest ecosystems in an aboveground–belowground context. Biological Invasions 19, 3301-3316.

Weil, R.R., Brady, N.C., Weil, R.R., 2016. The nature and properties of soils. Pearson.

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. American Journal of Alternative Agriculture 18, 3-17.

Weste, G., Marks, G.C., 1987. The biology of Phytophthora cinnamomi in australasian forests. Annual Review of Phytopathology 25, 207-229.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: Are there any links? Ecology 84, 2042-2050.

Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G., 1994. Functional diversity of microbial communities: A quantitative approach. Soil Biology and Biochemistry 26, 1101-1108.

Zhang, B., Zhou, X., Zhou, L., Ju, R., 2015. A global synthesis of below-ground carbon responses to biotic disturbance: a meta-analysis. Global Ecology and Biogeography 24, 126-138.

Supporting information

Table S1 Description of the studied sites. Modified from Ávila *et al.* (2017).

Variable	Open woodlands			Closed forests		
	S1	S2	S3	S1	S2	S3
Latitude (N)	36º 04′ 38″	36º 18' 37"	36º 31' 69"	36º 06' 09"	36º 23' 10"	36º 28' 13"
Longitude (W)	05º 33' 05"	05º 41' 14"	05º 38' 08"	05º 30' 53"	05º 31' 52"	05º 35' 31"
Precipitation (mm)	948.9	726.4	973.1	1067.1	1022.6	1097.0
Soil depth (cm)	35.82 ± 3.03	41.53 ± 3.50	45.02 ± 2.52	49.08 ± 2.27	57.24 ± 2.45	55.82 ± 2.35
Sand (%)	47.06 ± 1.75	68.60 ± 0.99	50.20 ± 1.66	72.90 ± 0.99	68.52 ± 1.04	75.26 ± 0.95
Silt (%)	22.01 ± 0.70	16.29 ± 0.49	17.92 ± 0.40	13.29 ± 0.44	17.78 ± 0.55	14.97 ± 0.53
Clay (%)	30.93 ± 1.30	15.12 ± 0.65	31.88 ± 1.51	13.81 ± 0.69	13.70 ± 0.68	9.77 ± 0.55
Ca (mg kg^{-1})	3397 ± 151	1455 ± 119	5181 ± 348	703 ± 39	961 ± 67	896 ± 57
K (mg kg^{-1})	261.6 ± 14.6	233.4 ± 10.4	299.5 ± 15.6	116.1 ± 6.3	154.5 ± 8.0	183.7 ± 11.4
N (mg g^{-1})	4.84 ± 0.30	3.53 ± 0.19	4.76 ± 0.27	2.49 ± 0.12	2.25 ± 0.14	2.27 ± 0.15
pH	5.94 ± 0.04	5.13 ± 0.03	5.92 ± 0.02	4.89 ± 0.05	5.04 ± 0.04	5.09 ± 0.04

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Table S2 Parameter estimates and 2-unit support intervals (in brackets) for the best model selected for each of soil variables in woodlands (H = Shannon-Weaver index, S = Simpson-Yule index, PC1 = first principal component, R_{mic} = microbial respiration, C_{mic} = microbial C, N_{mic} microbial N, Soil C = total soil C, DOC = dissolved organic carbon, Hex = hexoses, Pen = pentoses, AA= amino acids, Phe = phenols, DON = dissolved organic nitrogen, NH_4^+ and NO_3^-). Values of coefficients a_{site} and b are given. Different values of a_{site} coefficient indicate differences in background levels for different sites. ‡ = No differences were found between sites. Parameters α, β and λ of the neighbourhood index (NI – Eq. 3) are given (see text for details).

Variable	a_{Site1}	a_{Site2}	a_{Site3}	b	$\lambda_{heterospecific}$	$\lambda_{Q.\ suber}$	$\lambda_{Q.\ suber\ healthy}$	$\lambda_{Q.\ suber\ defoliated}$	$\lambda_{Q.\ suber\ dead}$	α	β
H	2.37 [2.33,2.42]	2.45 [2.40,2.50]	2.30 [2.56,2.37]	-	-	-	-	-	-	-	-
S	9.57 [9.09,10.07]	10.28 [9.76,10.78]	9.12 [8.66,9.61]	-	-	-	-	-	-	-	-
PC1	-0.04 [-0.06,-0.02]	0.45 [0.02,0.07]	0.001 [-0.02,0.02]	-	-	-	-	-	-	-	-
R_{mic}	11.87 [11.40,12.39]	7.58 [7.27,7.85]	9.80 [9.31,10.10]	0.16 [0.10, 0.22]	0.90 [0.18,1.00]	-	0.29 [0.08,0.61]	-0.97 [-1.00,-0.59]	0.19 [-1.00,1.00]	0.04 [0.00, 0.33]	1.50 [1.32, 2.02]
C_{mic}	-	1164.8 ‡ [1019.2,1317.8]	-	-	-	-	-	-	-	-	-
N_{mic}	-	115.9 ‡ [106.6,125.8]	-	-	-	-	-	-	-	-	-
Soil C	84.61 [80.38, 96.99]	78.89 [70.22,84.53]	85.39 [78.56,94.56]	0.25 [0.21,0.30]	-0.90 [-0.98,-0.38]	-	-0.48 [-0.51,-0.15]	0.05 [0.01,0.35]	0.25 [0.06,1.00]	1.55 [1.40, 1.90]	0.00 [0.00, 0.09]
DOC	435.5 [378.9,501.6]	318.6 [254.9,379,9]	208.5 [143.9,275.6]	-	-	-	-	-	-	-	-
Hex	39.72 [34.56,43.72]	6.45 [5.03,9.16]	15.09 [12.98,18.42]	5.26 [4.56,7.05]	1.00 [0.69,1.00]	-	0.82 [0.66,1.00]	-0.76 [-1.00,-0.38]	0.99 [0.60,1.00]	0.00 [0.00,0.11]	0.97 [0.82,1.06]
Pen	12.86 [11.19,14.86]	12.60 [11.09,14.72]	17.44 [15.00,20.43	-	-	-	-	-	-	-	-
AA	102.53 [100.48,104.51]	94.54 [92.65,96.43]	101.95 [99.92,103.93]	-	-	-	-	-	-	-	-
Phe	5.54 [4.82,6.25]	3.38 [2.98,3.88]	4.56 [4.02,5.41]	-	-	-	-	-	-	-	-
NH_4^+	24.76 [21.29,29.00]	5.88 [5.00,7.26]	20.88 [18.79,26.04]	293.3 [180.1,447.4]	0.12 [-0.81,1.00]	-	1.00 [0.63,1.00]	0.04 [-0.02,0.28]	-0.46 [-1.00,1.00]	2.29 [1.90,2.70]	3.51 [2.88,4.00]
NO_3^-	7.71 [7.25,9.37]	7.76 [7.37,9.68]	8.74 [7.95,10.75]	11.47 [8.13,13.31]	0.21 [-0.04,0.71]	-	-0.02 [-0.09,0.21]	-0.40 [-0.44,-0.23]	0.98 [0.81,1.00]	0.01 [0.00,0.20]	1.79 [1.64,2.00]
DON	28.73 [26.14,31.30]	20.57 [26.14,31.30]	22.49 [20.47,25.47]	-	-	-	-	-	-	-	-

Table S3 Parameter estimates and 2-unit support intervals (in brackets) for the best model selected for each of soil variables in closed forests (H = Shannon-Weaver index, S = Simpson-Yule index, PC1 = first principal component, R_{mic} = microbial respiration, C_{mic} = microbial C, N_{mic} microbial N, Soil C = total soil C, DOC = dissolved organic carbon, Hex = hexoses, Pen = pentoses, AA= amino acids, Phe = phenols, DON = dissolved organic nitrogen, NH_4^+ and NO_3^-). Values of coefficients a_{site} and b are given. Different values of a_{site} coefficient indicate differences in background levels for different sites. ‡ = No differences were found between sites. Parameters α, β and λ of the neighbourhood index (NI – Eq. 3) are given (see text for details).

Variable	a_{Site1}	a_{Site2}	a_{Site3}	*b*	$\lambda_{heterospecific}$	$\lambda_{Q.\ suber}$	$\lambda_{Q.\ suber}$ healthy	$\lambda_{Q.\ suber}$ defoliated	$\lambda_{Q.\ suber}$ dead	α	β
H	-	2.43 ‡ [2.40,2.45]	-	-	-	-	-	-	-	-	-
S	-	10.35 ‡ [10.04,10.65]	-	-	-	-	-	-	-	-	-
PC1	0.01 [-0.03,0.01]	-0.04 [-0.07,-0.02]	0.03 [0.00,0.05]	-	-	-	-	-	-	-	-
R_{mic}	7.78 [7.55,8.34]	7.76 [7.34,8.12]	6.28 [6.09,6.74]	0.19 [0.16, 0.23]	0.99 [0.79,1.00]	0.77 [0.59,1.00]	-	-	-	1.47 [1.31, 1.60]	0.67 [0.57, 0.74]
C_{mic}	-	645.1 ‡ [553.5,738.7]	-	-	-	-	-	-	-	-	-
N_{mic}	-	100.4 ‡ [91.4,109.3]	-	-	-	-	-	-	-	-	-
Soil C	24.42 [19.54,30.74]	17.45 [13.96,23.10]	28.20 [22.56,33.29]	92.70 [81.77,105.4]	1.00 [0.78,1.00]	-	0.99 [0.71,1.00]	0.90 [0.67,1.00]	0.06 [-0.33,0.50]	2.23 [2.10,2.36]	0.88 [0.82,0.94]
DOC	594.9 [511.7,659.8]	79.24 [70.52,105.0]	480.2 [437.0,578.6]	20.12 [15.43,22.89]	0.21 [0.12,0.29]	-	-0.39 [-0.43,-0.29]	-0.31 [-0.38,-0.14]	1.00 [0.91,1.00]	0.12 [0.06,0.26]	0.00 [0.00,0.10]
Hex	25.45 [21.38,28.25]	12.93 [10.34,17.55]	21.54 [16.37,23.68]	3.44 [2.04,4.41]	-0.06 [-0.17,0.03]	-	-0.41 [-0.56,-0.13]	-0.15 [-0.46,0.23]	0.99 [0.79,1.00]	0.00 [0.00,0.18]	0.49 [0.33,0.70]
Pen	4.92 [4.43,8.00]	8.46 [6.69,9.87]	5.89 [5.65,7.17]	3.61 [3.27,3.76]	1.00 [0.84,1.00]	-	1.00 [0.86,1.00]	-0.87 [-0.91,-0.74]	-0.74 [-0.85,-0.29]	0.14 [0.06,0.23]	1.08 [0.97,1.14]
AA	108.1 [106.0,110,1]	104.3 [102.3,106.3]	107.2 [105.0,110.2]	-	-	-	-	-	-	-	-
Phe	8.91 [8.02,10.24]	8.95 [7.61,9.89]	6.53 [5.75,7.37]	2.41 [1.91,2.87]	-0.63 [-1.00,-0.24]	-1.00 [-1.00,-0.74]	-	-	-	-	-
NH_4^+	16.66 [15.16,19.23]	16.87 [15.19,19.24]	21.98 [18.68,23.97]	0.47 [0.35,0.56]	0.67 [0.49,0.81]	0.07 [-0.05,0.23]	-	-	-	2.42 [2.18,2.77]	0.00 [0.00,0.14]
NO_3^-	5.45 [4.63,6.44]	4.10 [3.32,4.70]	3.07 [2.58,3.67]	45.21 [37.25,55.17]	-0.99 [-1.00,-0.79]	-0.20 [-0.30,-0.10]	-	-	-	3.98 [3.70,4.00]	2.24 [2.08,2.38]
DON	31.59 [27.48,35.58]	22.55 [18.49,26.54]	27.18 [23.10,31.17]	-	-	-	-	-	-	-	-

CAPÍTULO 5

PATHOGEN-INDUCED TREE MORTALITY INTERACTS WITH PREDICTED CLIMATE CHANGE TO ALTER SOIL RESPIRATION AND NUTRIENT AVAILABILITY IN MEDITERRANEAN SYSTEMS

Este capítulo reproduce el siguiente manuscrito:
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Pathogen-induced tree mortality interacts with predicted climate change to alter soil respiration and nutrient availability in Mediterranean systems

Resumen

Los ecosistemas de todo el planeta tienen que hacer frente simultáneamente a varios factores de cambio global con efectos potenciales importantes en el funcionamiento del ecosistema. Estos factores podrían interaccionar de forma inesperada, sin embargo, el conocimiento aun limitado que tenemos sobre estos efectos interactivos nos impide poder realizar predicciones del impacto del cambio global en el funcionamiento del ecosistema. En este trabajo se han evaluado los efectos directos de la mortalidad de árboles inducida por patógeno y el calentamiento y sequía sobre el C, N y P en suelos de bosques mediterráneos afectados por el decaimiento de su especie arbórea dominante (*Quercus suber*) debido al patógeno invasor *Phytophthora cinnamomi*. Además, se exploraron los potenciales efectos indirectos debido a la sustitución de especies tras la muerte de *Q. suber*. Para alcanzar el objetivo propuesto, se realizó un experimento de incubaciones en laboratorio a partir de suelos recogidos bajo individuos de *Q. suber* con diferente estado de salud (i.e. árboles sanos, defoliados y muertos) y de especies de matorral coexistentes (i.e. matorral pionero y de estados sucesionales más avanzados). Estos suelos fueron incubados bajo condiciones controladas de temperatura y humedad simulando varios escenarios predichos para 2050 y 2100 en la cuenca mediterránea. Los resultados de este trabajo mostraron que la mortalidad inducida por *P. cinnamomi* y el futuro calentamiento y sequía podrían interaccionar para alterar simultáneamente los ciclos biogeoquímicos en suelos de bosques de *Q. suber*. La resistencia de las variables estudiadas a cambios en la temperatura y la humedad tendieron a ser menores para suelos bajo árboles muertos que bajo árboles sanos y defoliados. Además, este trabajo demostró que tanto la respiración del suelo como la disponibilidad de nutrientes podrían ser afectados indirectamente por la sustitución de especies provocada por la mortalidad asociada a *P. cinnamomi*. En general, los resultados de este trabajo apoyan la idea del elevado potencial de las especies patógenas invasoras para modificar la respuesta del funcionamiento del suelo a factores de estrés de origen climático.

Abstract

Ecosystems worldwide must simultaneously cope with several global change drivers with potential strong effects on ecosystem functioning. These drivers might interact in unexpected ways, but our still limited understanding of these interactive effects precludes us from predicting the impact of global change on ecosystem functioning. In this study we assessed the direct effects of pathogen-induced tree mortality and predicted warming and drought on C, N and P in Mediterranean forest soils affected by the decline of their dominant tree species (i.e. *Quercus suber*) due to the invasive pathogen *Phytophthora cinnamomi*. We also explored the potential indirect effects due to species replacement after *Q. suber* mortality. To achieve our goal, we conducted a soil incubation experiment using soils collected under *Q. suber* trees with different health status (i.e. healthy, defoliated and dead trees) and from coexistent shrubs (i.e. pioneer and late successional shrubs). These soils were incubated under controlled temperatures and soil moistures, mimicking various climate change scenarios predicted for 2050 and 2100 in the Mediterranean Basin. Our results showed that *P. cinnamomi*-induced mortality and future warming and drought may interact to simultaneously alter biogeochemical cycles in *Q. suber* forest soils. Resistance of studied variables to changes in temperature and moisture tended to be lower for dead trees than for healthy and defoliated trees. Moreover, we found that soil respiration and nutrient availability might be affected indirectly by *P. cinnamomi*-induced mortality due to species replacement. Overall, our results support a high potential of invasive pathogen species for modifying the response of soil functioning to climatic stressors.

Keywords

Aridity, Climate Change, Drought, Forest disease, Global Change, Mediterranean shrubs, Non-additive effects, Oak decline, Pathogen, *Phytophthora cinnamomi*, *Quercus suber*, Soil nutrients, Soil respiration, Warming.

Introduction

Evidence of a strong effect of climate change on the structure and functioning of ecosystems have accumulated over the last decades (Cao & Woodward, 1998; Petchey *et al.*, 1999; Peñuelas *et al.*, 2002; Grimm *et al.*, 2013; Anderegg *et al.*, 2015). Many regions of the world are experiencing a general trend towards warmer temperatures and drier conditions (Giorgi & Lionello, 2008; IPCC, 2014b). These increasingly arid conditions might trigger significant changes in soil functioning in the following decades (Emmett *et al.*, 2004). For instance, it has been shown that predicted warming tends to increase carbon (C) and nutrient mineralization and nutrient availability as a consequence of higher microbial metabolism (Emmett *et al.*, 2004; Bai *et al.*, 2013), whereas drought usually reduces microbial activity and nutrient availability in soils (Emmett *et al.*, 2004; Matías *et al.*, 2012). However, other studies have found no effect of increased temperature (Dijkstra *et al.*, 2012; Auyeung *et al.*, 2013) or drier conditions (Auyeung *et al.*, 2013; Curiel Yuste *et al.*, 2014) on carbon mineralization and nutrient availability. These facts showed that the uncertainty associated with varying effects of warming and drought complicate forecasting predictions.

Besides climate change, ecosystems worldwide are faced with a variety of other global change-related drivers that affect ecosystem functioning such as invasive species, N deposition or changes in land-use (Vitousek, 1994; Trumbore *et al.*, 2015). More specifically, exotic pests and pathogens are considered a problem of paramount importance in forest systems, with profound direct and indirect impacts on plant communities and ecosystem functioning (e.g. Loo, 2009; Gandhi & Herms, 2010). Direct impacts, such as decrease in C stocks and soil respiration or increase in N availability, occur in the short-term (i.e. weeks to years) as a consequence of tree defoliation and mortality and the alteration of the abiotic and biotic environment under affected trees (Lovett *et al.*, 2006; Hicke *et al.*, 2012). Moreover, indirect impacts can occur in longer time scales (decades) due to species replacement as a consequences of differences among coexistent species in their vulnerability to mortality agents (Lovett *et al.*, 2010; Cobb *et al.*, 2013). The sign and magnitude of the indirect effects on biogeochemical cycles will depend on the differences between replacement species in their footprint on the soil environment (Lovett *et al.*, 2010; Cobb *et al.*, 2013).

Soils in forests affected by exotic pests or pathogens might cope with climate change stressors in unexpected ways. Multiple stressors (e.g. pests/pathogens, warming and

drought) may have simple additive effects on ecosystems. Alternatively, stressors might have interactive effects if one stressor increases or reduces the resistance of the ecosystem process to the other stressors (Paine *et al.*, 1998). These interactive effects lead to cumulative effects greater (synergistic interactions) or lower (antagonistic interactions) than the additive sum of the effects predicted from single-factor experiments. The single-factor approach therefore risks over- or under-estimating the impact that multiple drivers of global change might have on ecosystem process (e.g. Luo *et al.*, 2008; Morillas *et al.*, 2015). In the last decades much effort has been directed to analyze the impact of predicted climate change on soil. However, to our knowledge no previous studies have attempted to experimentally assess the effect of climate change on soil functioning and nutrient availability in forests affected by pest- or pathogen-induced tree mortality.

Mediterranean ecosystems are among those more threatened by global change (Giorgi & Lionello, 2008; Garbelotto & Pautasso, 2012). The Mediterranean Basin has suffered a strong increase in temperature (+1.5ºC) and drought severity (-15.6% precipitation; +7.4% evaporative demand) in the last five decades (Vicente-Serrano *et al.*, 2014). Moreover, Mediterranean systems are facing the consequences of invasive pests and pathogens that are severely altering ecosystems (Brasier, 1992; Garbelotto & Pautasso, 2012). For example, the exotic soil-borne pathogen *Phytophthora cinnamomi* is responsible for the severe decline of evergreen *Quercus* species (e.g. *Quercus suber* and *Quercus ilex*) in southern Europe (Sánchez *et al.*, 2002; Camilo-Alves *et al.*, 2013). Furthermore, the impacts of this pathogen may expand to other regions in the southern and northern hemisphere as a consequence of its potential range expansion under a warmer climate (Burgess *et al.*, 2017). Pathogen-driven tree mortality can have direct impacts on ecosystem functioning by reducing tree productivity, soil respiration rates and P availability, as well as by causing complex changes in the N cycle that are strongly context-dependent (Cherubini *et al.*, 2002; Shearer *et al.*, 2009; Ávila *et al.*, 2016). Moreover, gaps opened after tree death will be very likely occupied by coexistent drought-tolerant shrubs not affected by the pathogen (Ibáñez *et al.*, 2017), which might lead the system to a condition of arrested succession for at least several decades (Acácio *et al.*, 2007). The substitution of trees by shrubs might indirectly cause a substantial alteration of soil functioning as a consequence of the contrasting footprint of these two life forms on soils, with shrubs showing lower litter production and litter quality than trees (Rolo *et al.*, 2012). Comparing the effects of replacement shrubs and *Q. suber* trees on soil variables might help us to understand the indirect effect of tree mortality.

However, there is a lack of studies that have attempted to disentangle how Mediterranean forests disturbed by exotic pests or pathogens might direct or indirectly respond to increasing aridity, limiting our ability to predict the functioning of these systems under realistic global change scenarios.

In this study we aim to assess the effects of pathogen-driven tree mortality, warming and drought, and their interactions, on biogeochemical cycles in Mediterranean forests of southern Spain affected by the decline of their dominant species (i.e. *Q. suber*) due to the invasive pathogen *P. cinnamomi*. To achieve this goal, we conducted a soil incubation experiment using soils collected under *Q. suber* trees with different health status (i.e. healthy, defoliated and dead trees) and from coexistent shrubs (i.e. pioneer and late successional shrubs that occupy tree gaps after *Q. suber* death) that were incubated under controlled temperatures and soil moistures, mimicking various climate change scenarios predicted for the Mediterranean Basin. The specific objectives of this study were: 1) To analyze the direct effects of *P. cinnamomi*-driven tree mortality, predicted warming and drought and their interactions on soil respiration and nutrient availability (NH_4^+, NO_3^- and PO_4^{3-}); 2) To quantify the resistance (i.e. the amount of change caused by a disturbance, *sensu* Orwin and Wardle, 2004) of soil respiration and nutrient availability to predicted changes in temperature and soil moisture under trees with different health status; and 3) To explore the indirect effects of *P. cinnamomi*-driven tree mortality for soils under predicted warming and drought scenarios, due to the substitution of *Q. suber* trees by shrubs after tree death; we explored this by comparing the response to the climatic scenarios of soils under healthy *Q. suber* trees and successional shrubs.

Materials and methods

Study site

This study was conducted in mixed oak forests in Los Alcornocales Natural Park, southern Spain. The climate is sub-humid Mediterranean, characterized by warm and dry summers and cool, humid winters. Annual mean temperature ranges from 15.4 to 17.3 ºC and annual mean rainfall ranges from 970 to 1100 mm (period 1951-1999, Ninyerola *et al.*, 2005). Forests occur on acidic sandy soils interspersed with clay layers. The flora is dominated by mixed sclerophyll forests, with *Quercus suber* L. as the main species. In drier lowlands (hereafter open woodlands) *Q. suber* coexists with the evergreen and

shade-intolerant *Olea europaea* var. *sylvestris* Brot. and a dense and diverse understory dominated by shrub species such as *Pistacia lentiscus* L. and *Teline linifolia* L. The moister habitats (hereafter closed forests), are formed by *Q. suber* intermingled with the winter-deciduous shade-tolerant *Quercus canariensis* Willd. These closed forests are characterized by a rich understory dominated by shrub species such as *Arbutus unedo* L., *Ulex borgiae* Rivas Mart., *Erica arborea* L. and *Erica scoparia* L. (Ojeda *et al.*, 2000). In the study area, *Q. suber* adult trees show signs of defoliation and mortality. Extremely high abundances of *Phytophthora cinnamomi* Rands have been found in soils of symptomatic *Q. suber* trees, and therefore this pathogen has been suggested as a main driver of the species decline in the area (Gómez-Aparicio *et al.*, 2012).

Sampling design

Soil sampling was carried out during spring of 2012, the most biologically active season in Mediterranean-type ecosystems (García *et al.*, 2002). We selected two sites within the Natural Park, one in open woodlands (36º 4′N, 5º33′W) and another in closed forests (36º 23′N, 5º31′W). We included both forest types to increase the generality of our results and to explore the inherent heterogeneity that exists in the studied Mediterranean forests affected by *P. cinnamomi*. These forests are affected by a severe oak decline induced by *P. cinnamomi*, where 55 to 74% of *Q. suber* trees were categorized as defoliated or dead (Ávila *et al.*, 2017). In both forest sites, we selected soils from five different origins (hereafter soil origin) to analyze the direct and indirect effect of *P. cinnamomi*-induced tree mortality on soil variables: soils under *Q. suber* trees with different health status (i.e. healthy, defoliated and dead) and under pioneer and late-successional shrubs. At each site, we selected 5 individual trees of average size (i.e. 30-40 cm diameter at breast height) per health category. We also selected five individuals of each of the two most abundant shrub species at each forest type belonging to two different successional stages: *Teline linifolia* (pioneer) and *Pistacia lentiscus* (late-successional) in the open woodland, and *Ulex borgiae* (pioneer) and *Erica arborea* (late-successional) in the closed forest (See Figure S1 in Supplementary Material). We sampled soil under trees and shrubs avoiding overlapping crowns with individuals of different species or health status. Soil samples were collected within two meters from the bole of each individual tree and under the canopy of the shrubs. The samples were taken from the top 15 cm of the soil using a circular soil corer (5 cm diameter x 20 cm height). Each soil sample (2 sites x 5 soil

origins x 5 individuals = 50 samples) was transported in polyethylene bags in coolers to the laboratory, and further maintained as independent samples (Reinhart & Rinella, 2016). Soil was sieved at 2 mm and air-dried at room temperature until a constant weight was obtained (~ 4 weeks). Prior to soil incubations, we determined the water holding capacity (WHC) of each soil sample using a subsample of 50 g that was saturated by water, covered and allowed to drain for 48 h. The water content of the drained soil was considered 100% of the WHC (Agehara & Warncke, 2005). Soil gravimetric water content was determined by oven drying samples at 105º C for 24 h.

Soil incubations: temperature and soil moisture scenarios

In order to simulate the effect of predicted warming and drought on soil variables, we selected different temperatures and soil moistures based on projections of climatic models. For temperature, we used three different scenarios. The first one (T = 16ºC) is considered the mean spring temperature in the study sites (Ninyerola et al., 2005). The other two scenarios (i.e. 19 and 22ºC) were the projected spring mean temperatures for the study site for 2040-2070 and 2070-2100, respectively (Moreno & Obrador, 2007). These projections were determined from the HadCM3 (an atmosphere-ocean coupled general circulation model, ACGCM) using the scenario SRES A2, the most common scenario from IPCC (Nakicenovic & Swart, 2000). For soil moisture, we chose 4 different scenarios of soil water content (SWC) in terms of WHC: 80%, 40%, 32% and 15% of the WHC. The first scenario was 80% of the WHC simulating a wet spring, where soils remain saturated most of the time (as it was observed in 2010 at our study sites, J.M. Ávila, unpublished data). We did not use 100% of WHC to avoid problems with gas diffusion in soil respiration measurements. The second scenario was 40% of the WHC, which is the average soil moisture in spring for these sites (period 2010-2012; J.M. Ávila, unpublished data). The third scenario was a reduction of 20% in soil moisture predicted for 2050 (the same period than for the second scenario of temperature) using an ACGCM for the scenario SRES IS92a (Wetherald & Manabe, 2002; Manabe *et al.*, 2004). We used this scenario because it is comparable with the SRES A2 (Wang *et al.*, 2004) and, as far as we know, it is the only one that has been used to make predictions in soil moisture. Soil moisture under this scenario was 32% of WHC. Finally, the last scenario was a simulation of an extremely dry spring (15% of WHC). We used soil moisture data from spring of 2012 as a reference because that year the precipitation was the lowest in the last 50 years (following data from the meteorological station Jerez de la Frontera Airport, 36°

45' 2" N and 6º 3' 20" W) and, specifically, the spring rainfall in the study area was extremely low (60% lower than in a normal year, 1951-1999, Ninyerola *et al.*, 2005).

Air-dried soil samples (20 g) from the different five soil origins (i.e. soils underneath healthy, defoliated and dead *Q. suber*, as well as under pioneer and late successional shrubs) were introduced in polypropylene specimen cups and were incubated during 4 weeks using the different scenarios of temperature and soil moisture (TEM = 16, 19 and 22ºC; SWC = 80, 40, 32, 15% of WHC) following a factorial design (2 forest sites, 5 levels of soil origin, 3 levels of TEM, 4 levels of SWC, 5 replicates per treatment combination = 600 incubations). This 4-week incubation experiment represents a short-term assay that can estimate microbial activity and C, N and P fluxes rapidly and reliably (Franzluebbers, 1999). Soil samples were incubated in growth chambers in dark conditions. The cups were closed with polyethylene film allowing gas exchange, but limiting water losses. Moisture was monitored weekly by weighing and it was corrected when reduced >5%.

Lab analyses

For each soil sample, we monitored C mineralization measuring CO_2 produced by soil microorganisms using a colorimetric method (García-Palacios *et al.*, 2013). Once per week, one 4-well microstrip filled with cresol red agar (indicator) was attached to the cup side. Cresol red agar is a mixture of an indicator solution (18.75 ppm cresol red dye, 220 mM potassium chloride and 3.75 mM sodium bicarbonate) amended with melted 3% purified agar (2:1 indicator:agar). The jars were closed air tight for 6 hours and the absorbance of the microsptrips was read at 595 nm before and after that period. The well absorbance after the 6-hour period was normalized, averaged in each jar and converted to CO_2 concentration using a calibration curve created for this method in the lab (Campbell et al., 2003). We calculated cumulative respiration over the entire length of the incubation (i.e. from the 4 weekly measures). To do this, we previously computed for each cup the average soil respiration rate per week (Milla *et al.*, 2006).

For each soil sample we estimated N availability using ionic exchange membranes (Subler *et al.*, 1995; Qian & Schoenau, 2002; Durán *et al.*, 2013). We chose this method because previous studies have demonstrated that it is an excellent approach for estimating soil nutrient availability, showing a strong correlation with plant nutrient uptake (Schoenau & Huang, 1991; Ziadi *et al.*, 1999). Anion and cation exchange membranes (types I-100 and I-200, Electropure Excellion, Laguna Hills, California) of 2.5 x 2.5 cm

were incubated together with the soil samples during 4 weeks. After the incubation, the adsorbed nutrients in the membranes were extracted using 25 ml of 2 M KCl in 125 ml flasks by orbital spinning for 1h at 200 rpm. Ammonium and nitrate concentrations were assessed with the indophenol-blue method (Sims *et al.*, 1995).

P availability was determined as the labile inorganic fraction of P, and it was analyzed after soil incubation using ionic membranes following the method described by Schoenau and Huang (1991) and Kouno *et al.* (1995). A moist soil sample of 2 g was placed in a 50 ml centrifugation flask with an anionic exchange membrane (2.5 x 2.5 cm squares). Then 20 ml of distilled water was added, and the flasks were shaken on an orbital shaker for 16 hours. After extraction, the adsorbed phosphorus in the membranes was extracted using 15 ml of 0.5M HCl for 1 h added in 125 ml flasks by orbital spinning for 1 h at 200 rpm. Inorganic P in the extraction eluents was analyzed by the molybdenum-blue method (Allen *et al.*, 1986).

Data analyses

Generalized Linear Models (GLM) were used to test for the direct (Objective 1) and indirect (Objective 3) effects of pathogen-induced tree mortality, the predicted increase of temperature (TEM, three levels) and the decrease of soil moisture (SWC, four levels), as well as all possible interactions among those factors on the different studied variables. Data for the two forest types were analyzed separately. To address whether tree health, warming, drought and their interactions impact on soil variables (Objective 1), we used data for the three health states of *Q. suber* (HEALTH, three levels). To explore the indirect effects of tree mortality as a consequence of species substitution (Objective 3), we used data for soils underneath healthy *Q. suber* and the different shrubs (SPE, three levels). Including interaction terms within the models allowed testing for deviation from the additive model and the identification of antagonistic and synergistic interactions among factors (i.e. interactive, Piggott *et al.*, 2015). We fit GLMs using a gamma family with identity as the link function. We chose a gamma distribution because our response variables were not normally distributed and it provided a better fit than the normal or lognormal distribution. Backward model selection was applied starting from the saturated model that included all the factors and their interactions. We removed terms (starting by the interactions) until there was no further decrease in the Akaike Information Criterion (AIC, Burnham & Anderson, 2002). Two nested models were considered as equivalent in terms of fit when they did not differ in more than 2 AIC units. When significant

interactions between factors were found, *post hoc* tests (Tukey test) were performed to determine which levels of the independent factor were significantly different (Crawley, 2007). Model assumptions were verified by examination of residuals (McCullagh & Nelder, 1989).

To evaluate how tree mortality affects the resistance of soil processes to predicted changes in temperature and soil moisture (Objective 2), we calculated a Resistance Index (RS, Orwin & Wardle, 2004) for the studied variables (i.e. soil respiration, NH_4^+ and NO_3^- and P availability):

$$RS_i = 1 - \frac{2|D_i|}{(C_i + |D_i|)}$$

where D_i is the difference between the control (C_i) and the disturbed soil at the end of the disturbance for each soil sample (i). To calculate this index, we considered two scenarios of disturbance using data of soils incubated in two combinations of temperature and soil moisture: Scenario 2050 and Scenario 2100. For Scenario 2050 we considered the increase in temperature and decrease in soil water content predicted by climate models for 2050 (19ºC and 32% of WHC), whereas in Scenario 2100 we used the predicted temperature and an extremely dry spring for 2100 (22ºC and 15% of WHC, see scenarios above). We considered as reference the soils incubated at the mean spring temperature (16ºC) and the average soil moisture in spring (40% of WHC). This index of resistance is bounded between -1 and +1, with a value of -1 showing less resistance and +1 maximal resistance. We fit GLMs using a Gaussian family with identity as the link function. Due to the bounded nature of the index, data were cube-root or fifth-root transformed when necessary to meet the assumptions of the models (Cox, 2011). Model selection was based on a backward procedure using AIC as before. Multiple comparison using Tukey tests were performed to determine statistical differences among *Q. suber* health status and scenarios (Crawley, 2007). All statistical analyses were performed using R 3.1.1 software (R Core Team, 2014) and figures were created using Sigmaplot v. 12 (Systat Software 2011).

Results

Direct effects of pathogen-driven tree mortality, warming and drought on soil respiration and nutrient availability

Our first objective was to address the effects of tree health, warming and drought on the studied soil variables. Our models showed that soil respiration was affected by the three studied factors, i.e. soil origin (i.e., soils collected under *Q. suber* trees with different health status), soil moisture and temperature (Table 1). However, soil moisture was the best predictor of soil respiration variability (i.e. the factor with the highest explained deviance in each model, Table 1). In the open woodland, the effects of *Q. suber* decline and the reduction in soil moisture on soil respiration were negative (Fig. 1a). We did not detect an effect of predicted warming on soil respiration (Table 1, Fig. 2a). Meanwhile, in the closed forests, we detected an interaction between soil origin and soil moisture. The reduction of soil respiration as soil moisture decreased from 80 to 32% of WHC was of higher magnitude in soils collected under dead trees (29%) than under healthy (19%) or defoliated trees (8%). A positive effect of *Q. suber* decline was detected when soil moisture decreased at 40 and 15% of WHC (Fig. 1e). The predicted warming led to an increase in soil respiration independently of the soil origin (Table 1, Fig 2e), but this positive effect on soil respiration was only significant at the higher soil moisture treatment (Table 1, see Fig S2 in the Supplementary Material). In both forest types, the simulation of an extremely dry spring (15% of WHC) strongly reduced soil respiration to similar values in all soil origins (Fig. 1a, e).

The availability of NH_4^+ and NO_3^- was strongly affected by soil origin and soil moisture in both forest types, whereas the effect of temperature was only detected for NH_4^+ (Table 1). Soil moisture was the factor that most explained the variance in N availability (Table 1). We detected significant interactions between the effects of soil origin and soil moisture in both forest types (Table 1). The reduction in N availability with decreasing soil moisture from 80 to 32% of WHC was generally of higher magnitude in soils collected under defoliated and dead trees than under healthy trees (Fig. 1b, c, f). We mostly detected a complete reduction in N availability in the extreme moisture treatment (i.e. 15% of WHC, Fig 1b, c, f, g). We found a significant positive effect of predicted temperature on NH_4^+ in open woodlands, while in closed forests the positive effect of temperature was of higher magnitude in soils collected under dead trees (95%)

than under defoliated (-8%) or healthy trees (32%) (Fig 2b, c, f, g). A significant interaction between temperature and soil moisture was found for NH_4^+ in closed forests. The positive effect of temperature was detected only for 32% of WHC (Table 1, see Fig S3 in the Supplementary Material).

Soil origin, soil moisture and temperature all contributed to explain PO_4^{3-} variability in both forest types (Table 1). We found that defoliated trees had lower PO_4^{3-} availability than healthy trees (Fig. 1d, h and 2d, h). However, in open woodlands, this effect disappeared as temperature increased (interactive effect between soil origin and temperature, Table 1, Fig. 2d). We also detected strong interactions between temperature and soil moisture on PO_4^{3-} availability in both forest types, with the negative effect of temperature disappearing as soil moisture decreased (Table 1, Fig. 3).

Resistance of soil respiration and nutrient availability to predicted future scenarios

We analyzed the resistance of soil respiration and nutrient availability in soils collected under *Q. suber* trees with different health status to changes in soil moisture and temperature in two future climatic scenarios (Objective 2, Table 2, Fig. 4). Resistance of soils to changes in soil respiration and nutrient availability tended to be lower for dead trees than for healthy and defoliated trees (Fig. 4). We detected significant differences between soil origins in their resistance to changes in soil respiration in the closed forest, and in NO_3^- and PO_4^{3-} availability in the open woodland (Fig. 4). We also found marginal significant differences between soil origins in the resistance of soils to changes in NH_4^+ availability in the open woodland and in PO_4^{3-} availability in the closed forest (Table 2, Fig. 4). Moreover, we detected a reduction in soil resistance in the extreme scenario (2100) compared to the conditions predicted for 2050 for soil respiration (WL and CF, Tukey test $SC_{2050}>SC_{2100}$, Tukey $p<0.05$) and N availability (NH_4^+ in WL, Tukey test $SC_{2050}>SC_{2100}$ $p<0.05$; NO_3^- in WL and CF, Tukey test $SC_{2050}>SC_{2100}$ $p<0.05$), but we did not detect a significant effect of scenarios for P availability (Table 2).

Table 1 Analysis of deviance for the effects of soil origin (HEALTH: healthy, defoliated and dead tree), temperature (TEM: 16, 19 and 22ºC) and soil moisture (SWC: 80, 40, 32 and 15% WHC) on soil respiration and nutrient availability in woodland (WL) and closed forest (CF). Values of degrees of freedom (d.f.), deviance (Dev.) and F statistic (F) are presented. Dash indicates a factor not included in the minimum adequate model.

Forest	Source	CO_2			NH_4^+			NO_3^-			PO_4^{3-}		
		d.f.	Dev.	F	d.f.	Dev.	F	d.f.	Dev.	F	d.f.	Dev.	F
WL	NULL		18.86			825.93			352.82			91.32	
	HEALTH	2	0.55	8.85***	2	28.62	22.06***	2	3.02	3.42*	2	11.92	23.32***
	TEM		-		2	6.53	5.03**	2	1.81	2.05$^{n.s.}$	2	9.36	18.32***
	SWC	3	12.81	148.30***	3	465.83	239.39***	3	207.11	156.51***	3	9.98	13.02***
	HEALTHxTEM		-		4	3.51	1.35$^{n.s.}$		-		4	2.63	2.58*
	HEALTHxSWC		-		6	18.37	4.72***	6	70.15	26.51***	6	3.1	2.02$^{n.s.}$
	TEMxSWC		-			-			-		6	9.91	6.46***
	Residual	174	5.51		162	303.08		166	70.74		156	44.431	
CF	NULL		11.93			925.26			1421.7			766.99	
	HEALTH	2	0.31	6.35**	2	33.67	25.95***	2	56.16	21.88***	2	33.00	7.22***
	TEM	2	0.24	4.93**	2	4.58	3.53*	2	5.43	2.12$^{n.s.}$	2	190.82	41.77***
	SWC	3	6.50	89.22***	3	568.46	292.16***	3	604.07	156.87***	3	93.55	13.65***
	HEALTHxTEM		-		4	56.99	21.97***	4	11.94	2.33$^{n.s.}$		-	
	HEALTHxSWC	6	0.31	2.13*	6	26.42	6.79***	6	113.93	14.79***		-	
	TEMxSWC	6	0.59	4.07***	6	23.29	5.99***		-		6	70.42	5.14***
	Residual	160	3.97		156	211.85		162	640.15		166	379.21	

*** p<0.001, ** p<0.01, *p<0.05, n.s. non significant

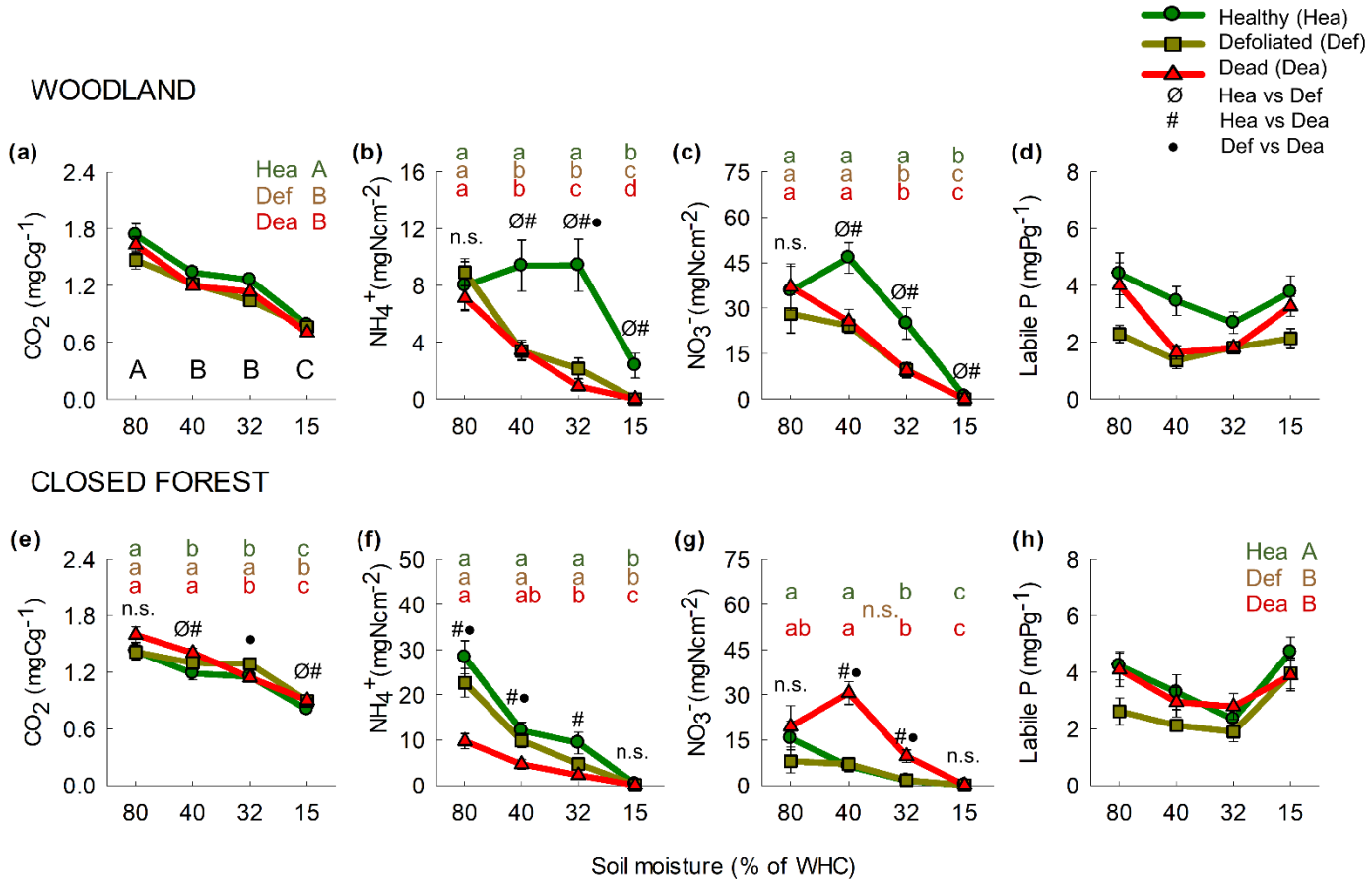

Figure 1 Soil respiration (a, e) and NH_4^+ (b,f), NO_3^- (c, g) and PO_4^{3-} availability (d,h) (mean ± SE) for each soil origin (soils under healthy, defoliated and dead *Q. suber*) at each soil moisture level (n=15). Graphics in the first row represent soils from the woodland and graphics in the second row represents soils from the closed forest. The lower cases indicate significant differences ($p<0.05$) in the post-hocs analyses: if interactive effects between soil origin and soil moisture were significant, symbols represent differences in the soil variables between soil origin within SWC levels and colored letters indicate significant differences in the soil variables between SWC within soil origin (top letters for HEA, mid-letter for DEF and bottom letter for DEA); if interactive effects between soil origin and soil moisture were not significant, upper case letters indicate differences between levels of the main effects.

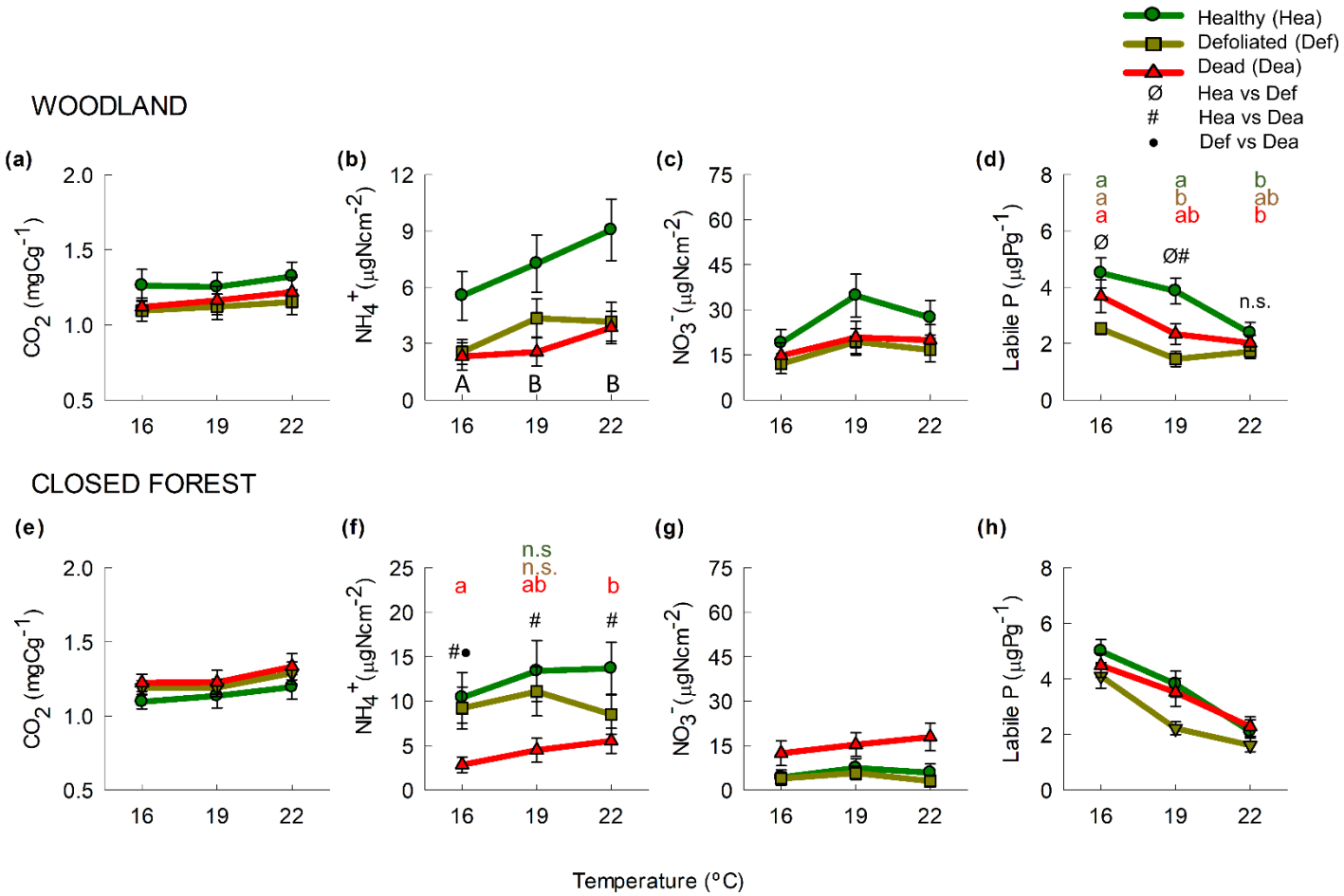

Figure 2 Soil respiration (a, e) and NH_4^+ (b,f), NO_3^- (c, g) and PO_4^{3-} availability (d,h) (mean ± SE) for each soil type at each temperature (n=20). Graphics in the first row represent soils from woodland and graphics in the second row represents soils from closed forests. The lower cases indicate significant differences ($p<0.05$) in the post-hocs analyses: if interactive effects between soil origin and temperature were significant, symbols represent differences in the soil variables between soil origin within temperature levels and colored letters indicate significant differences in the soil variables between temperature within soil origin (top letters for HEA, mid-letter for DEF and bottom letter for DEA); if interactive effects between soil origin and temperature were not significant, upper case letters indicate differences between levels of the main effects.

Table 2 Analysis of variance for the effect of soil origin (HEALTH: healthy, defoliated and dead tree) and two different future scenarios (SC: 2050 and 2100) on the Resistance Index (RS) of soil respiration and nutrient availability in open woodland (WL) and closed forest (CF). Values of degrees of freedom (d.f.), mean square (MS) and F statistic (F) are presented. The results of the interaction between HEALTH and SC are not shown because it was not significant in any case. Dash indicates a factor not included in the minimum adequate model.

Forest	Source	CO_2 RS			NH_4^+ RS			NO_3^- RS			PO_4^{3-} RS		
		d.f.	MS	F	d.f.	MS	F	d.f.	MS	F	d.f.	MS	F
WL	HEALTH		-		2	0.596	2.519 •	2	0.092	5.29*	2	0.370	4.74*
	SC	1	0.853	47.03***		-		1	2.460	142.04***		-	
	Residual	28	0.508					26	0.017		27	1.014	
CF	HEALTH	2	0.053	4.52*		-			-		2	0.29	2.98 •
	SC	1	0.406	34.14***	1	2.496	61.14***	1	1.173	27.04***		-	
	Residual	26	0.012		28	0.041		28	0.064		27	0.098	

*** p<0.001, ** p<0.01, *p<0.05, •p<0.10, n.s. non significant

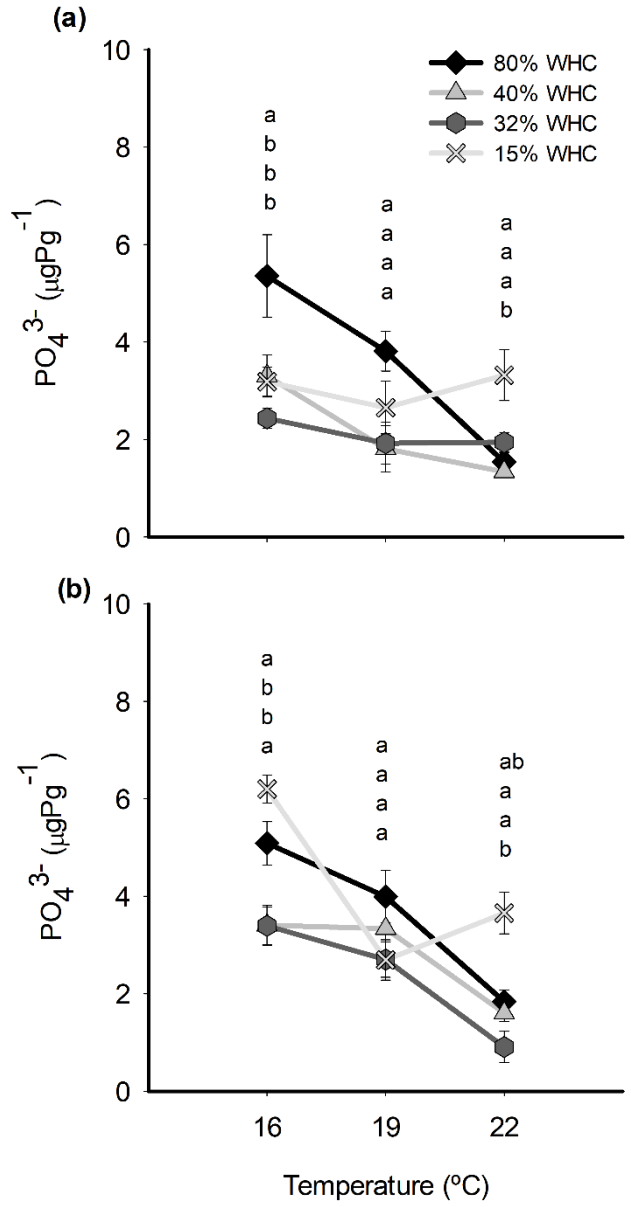

Figure 3 PO_4^{3-} availability (mean ± SE) for each soil temperature at each soil moisture level (n=15) in a) woodland and b) closed forest. The lower case indicates significant differences in PO_4^{3-} availability between temperature levels within moisture levels ($p<0.05$).

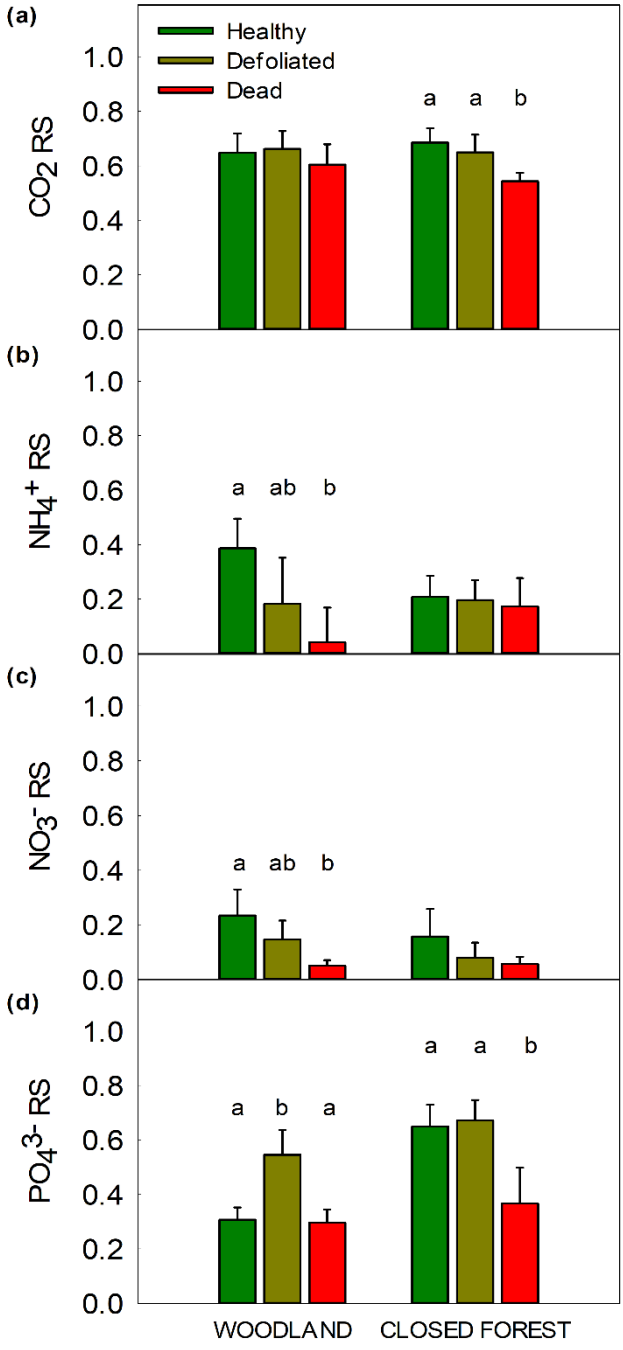

Figure 4 Changes in the Resistance Index (RS) for microbial respiration (a), NH_4^+ (b), NO_3^- (c) and PO_4^{3-} (d) availability under predicted changes in climate induced by climate change for different soil types in woodland and closed forests. Data are means ± SE (n=10). Data were pooled among climate change scenarios because we did not detect a significant interaction between scenario and soil type. The lower case indicates significant differences in RS between health status of trees ($p<0.05$).

Indirect effects of pathogen-driven tree mortality, warming and drought on soil respiration and nutrient availability

When we analyzed the potential indirect effects of *Q. suber* mortality on soil respiration and nutrient availability (Objective 3), our models revealed significant interactive effects between soil origin (i.e. *Q. suber*, pioneer and late-successional shrubs) and soil moisture and temperature (Table 3). We found a negative effect of drought (from 80 to 32% of WHC) on soil respiration in soils under healthy *Q. suber* trees and late-successional shrubs (Fig. 5a,e), but a positive effect of temperature on soil respiration in all soil origins. We found complex interactive patterns on the effect of soil origin, soil moisture and temperature on N availability, showing a high level of context-dependency. In the open woodland, we found a negative effect of soil moisture (from 80 to 32% of WHC) on N availability for pioneer shrubs but no effect for healthy *Q. suber* trees. However, in closed forests, we detected a negative effect of drought (from 80 to 32%WHC) on N availability for healthy trees but not for pioneer shrubs (Fig. 5b, c, f, g). We detected a positive effect of predicted warming on NO_3^- (in open woodlands) and NH_4^+ (in closed forests) just for pioneer shrubs (Fig. 6b, c, f, g). In general, we detected lower PO_4^{3-} availability under pioneer shrubs than under healthy *Q. suber* and late-successional shrubs. We found a negative effect of predicted drought (from 80 to 32% of WHC) on PO_4^{3-} availability. In the closed forest this effect was of higher magnitude in soils collected under pioneer shrubs than under healthy *Q. suber* trees. The negative effect of temperature on PO_4^{3-} availability was of higher magnitude for shrubs than for *Q. suber* trees (SPExTEM, $p<0.05$). In both forest types, the simulation of an extremely dry spring (15% of WHC) strongly reduced soil respiration and N availability to similar values in all soil origins (Fig. 5).

Table 3 Analysis of deviance for the effects of soil origin (SPE: *Q. suber*, pioneer shrub and late-successional shrub), temperature (TEM: 16, 19 and 22ºC) and soil moisture (SWC: 80, 40, 32 and 15% WHC) on soil respiration and nutrient availability in woodland (WL) and closed forest (CF). Values of degrees of freedom (d.f.), deviance (Dev.) and F statistic (F) are presented. Dash indicates a factor not included in the minimum adequate model.

Forest	Source	CO_2			NH_4^+			NO_3^-			PO_4^{3-}		
		d.f.	Dev.	F	d.f.	Dev.	F	d.f.	Dev.	F	d.f.	Dev.	F
WL	NULL		16.95			290.97			841.17			114.44	
	SPE	2	0.41	7.93***	2	14.53	15.29***	2	2.87	$1.62^{n.s.}$	2	14.98	23.68***
	TEM	2	0.18	3.51*	2	1.40	$0.97^{n.s.}$	2	11.28	6.39**	2	14.76	23.32***
	SWC	3	11.56	148.19***	3	77.87	35.81***	3	524.08	197.90***	3	9.62	10.13***
	SPExTEM		-		4	-		4	10.84	3.07*	4	2.83	$2.23^{n.s.}$
	SPExSWC	6	0.51	3.27**	3	12.53	2.88*	6	14.99	2.83*	6	3.47	$1.83^{n.s.}$
	TEMxSWC		-			-		6	12.71	2.40*	6	19.79	10.42***
	Residual	166	4.28		162	184.64		156	264.41		156	49.00	
CF	NULL		11.57			970.65			1681.78			146.26	
	SPE	2	1.07	18.85***	2	13.32	9.59***	2	147.88	62.20***	2	3.71	5.43**
	TEM	2	0.37	6.48**	2	22.7	16.33***	2	54.23	22.81***	2	28.83	42.21***
	SWC	3	4.47	52.40***	3	565.57	271.32***	3	262.98	73.74***	3	12.64	12.34***
	SPExTEM		-		6	51.94	18.69***		-		4	9.10	6.66***
	SPExSWC	6	0.47	2.78*	4	24.99	5.99***	6	445.59	62.47***	6	12.32	6.01***
	TEMXSWC	4	0.66	3.88**		-		6	117.39	16.46***	6	5.01	2.45*
	Residual	162	4.53		162	292.14		160	653.71		156	74.66	

*** p<0.001, ** p<0.01, *p<0.05, n.s. non significant

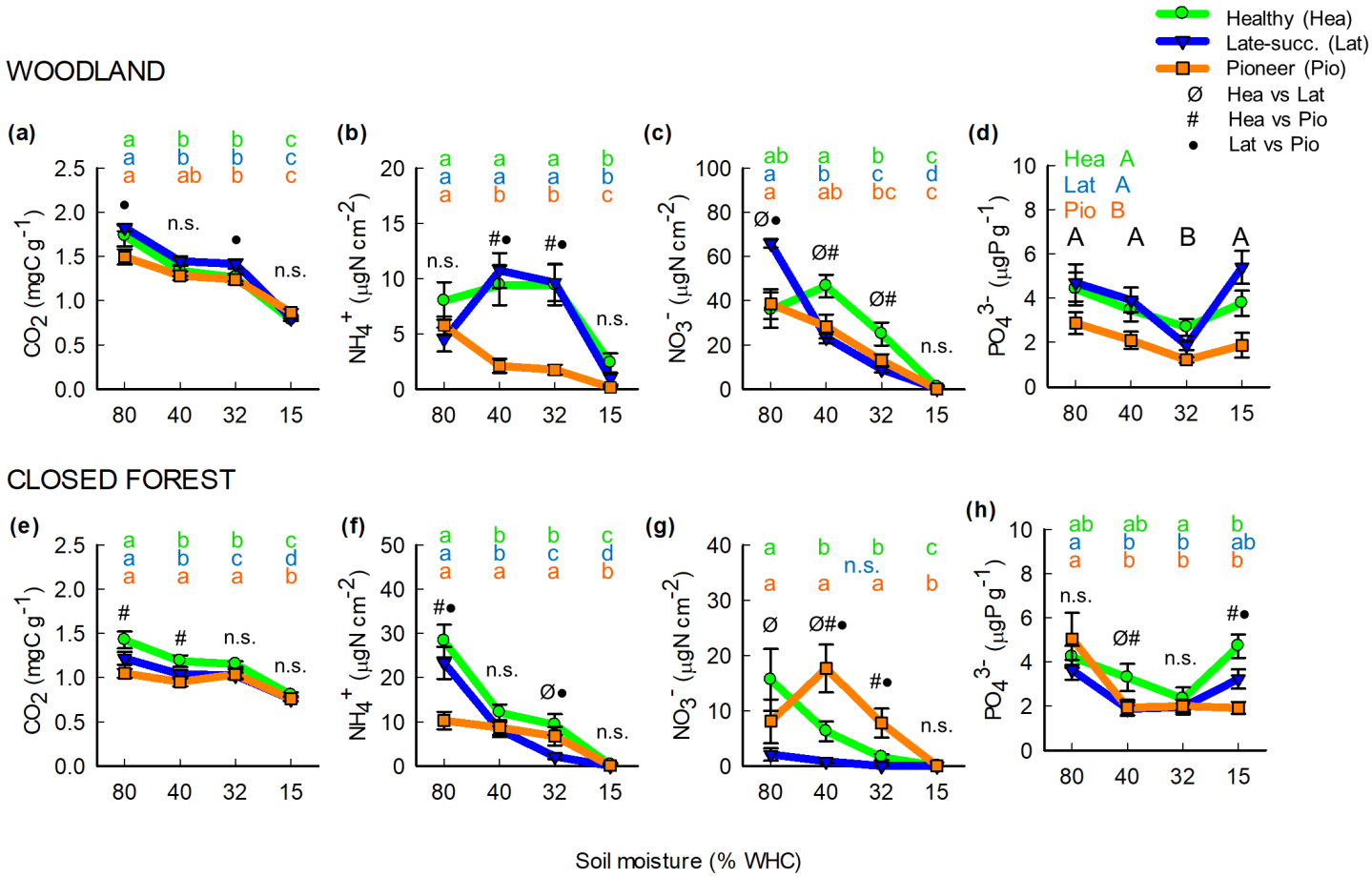

Figure 5 Soil respiration (a, e) and NH_4^+ (b,f), NO_3^- (c, g) and PO_4^{3-} availability (d,h) (mean ± SE) for each soil origin (soils under healthy *Q. suber*, late-successional and pioneer shrubs) at each soil moisture level (n=15). Graphics in the first row represent soils from the woodland and graphics in the second row represents soils from the closed forest. The lower cases indicate significant differences ($p<0.05$) in the post-hocs analyses: if interactive effects between soil origin and soil moisture were significant, symbols represent differences in the soil variables between soil origin within SWC levels and colored letters indicate significant differences in the soil variables between SWC within soil origin (top letters for HEA, mid-letter for DEF and bottom letter for DEA); if interactive effects between soil origin and soil moisture were not significant, upper case letters indicate differences between levels of the main effects.

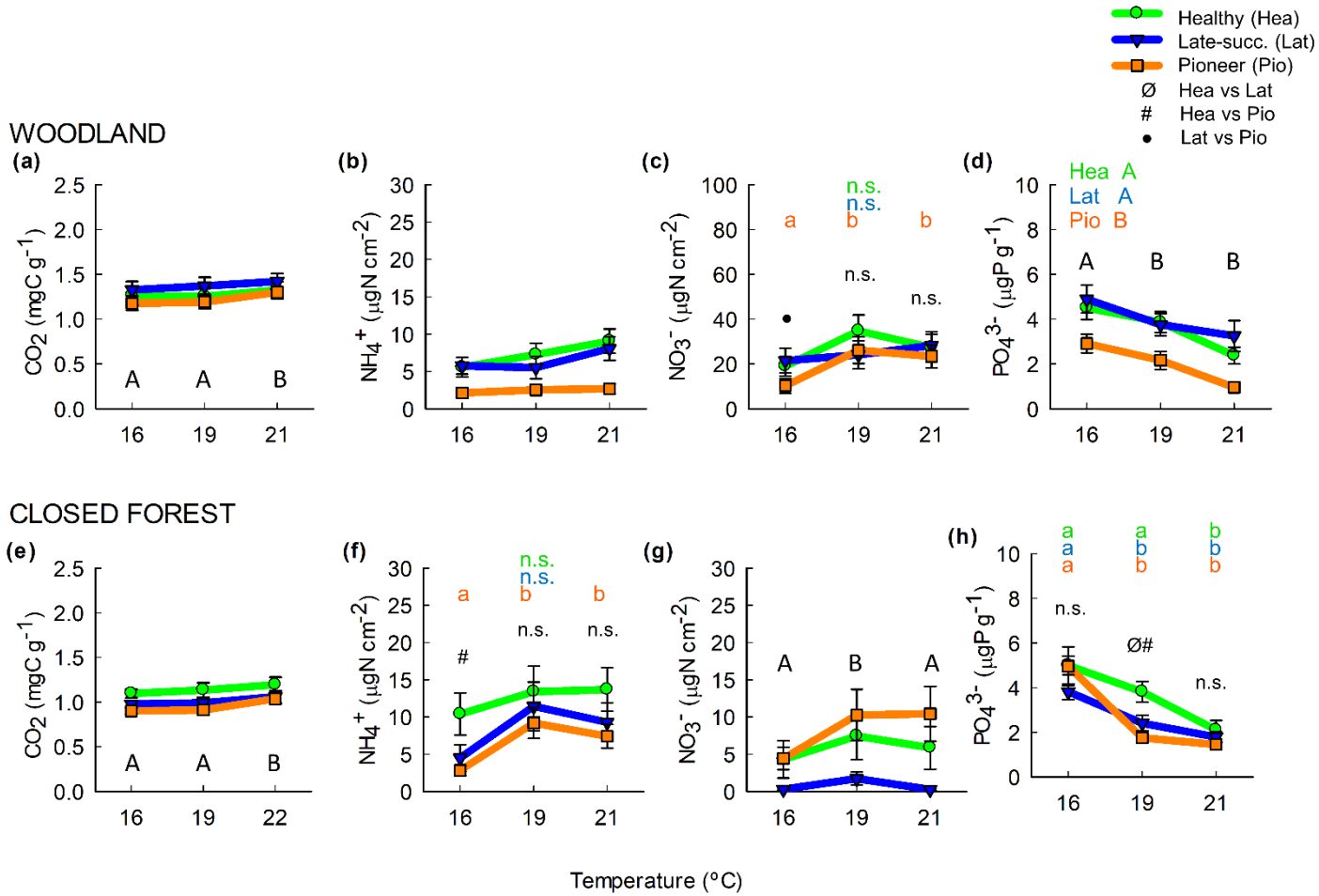

Figure 6 Soil respiration (a, e) and NH_4^+ (b,f), NO_3^- (c, g) and PO_4^{3-} availability (d,h) (mean ± SE) for each soil origin (soils under healthy *Q. suber*, late-successional and pioneer shrubs) at each temperature (n=20). Graphics in the first row represent soils from the woodland and graphics in the second row represents soils from the closed forest. The lower cases indicate significant differences ($p<0.05$) in the post-hocs analyses: if interactive effects between soil origin and temperature were significant, symbols represent differences in the soil variables between soil origin within temperature levels and colored letters indicate significant differences in the soil variables between temperature within soil origin (top letters for HEA, mid-letter for DEF and bottom letter for DEA); if interactive effects between soil origin and temperature were not significant, upper case letters indicate differences between levels of the main effects.

Discussion

This study provides useful insights into how multiple global change drivers affect soil functioning in Mediterranean ecosystems. Our models demonstrated that *P. cinnamomi*-induced mortality and future warming and drought may interact to simultaneously affect biogeochemical cycles in *Q. suber* forest soils. Moreover, we found that in a context of pathogen-driven mortality, soils under affected trees had lower resistance to the warming and drought scenarios (predicted for 2050 and 2100) than soils under healthy trees, consistently in both forest types. Previous works have warned about the consequences of future climate conditions on soil functioning (Emmett *et al.*, 2004; IPCC, 2014a), but interactive effects of future climatic conditions and biotic disturbances have been rarely experimentally addressed so far. This study represents an effort to fill this gap by exploring how the effect of predicted climate change might differ in soils affected by biotic disturbances, using a manipulative lab experiment in ways that are not feasible to address in the field.

Direct effects of pathogen-tree mortality, warming and drought on soil variables

Our results indicate that the predicted drought would translate into a reduction in soil respiration and N availability in both forest types. However, the magnitude of such decrease varied strongly depending on tree health, as indicated by the interactive effects of soil origin and soil moisture in most of our best models. For example, in the open woodland the magnitude of the change in N availability as a consequence of increasing drought (from 80 to 32% of WHC) was much higher in soils collected under dead (-85% for NH_4^+ and -70% for NO_3^-) and defoliated trees (-73% for NH_4^+ and -59% for NO_3^-) than under healthy trees (+18% for NH_4^+ and -30% for NO_3^-). This result suggests synergistic interactions between stressors, since the cumulative effect of pathogen-induced mortality and drought was of higher magnitude than the additive sum of the effects of both factors. The interactions found here between the effects of *Q. suber* decline and reduced soil moisture exemplify how invasive pathogen species have a high potential for modifying the response of soil functioning to climatic stressors.

Extreme climatic events like droughts can drastically alter plant and microbial communities and soil functioning (Wang *et al.*, 2012; Lloret *et al.*, 2015). These extreme events may be even more critical drivers of ecosystem functioning than changes in mean

conditions (Heisler & Weltzin, 2006; IPCC, 2014a). In our study, we found a large reduction in soil respiration and N availability when soils experienced an extremely dry spring in both forest types. This high level of water stress reduced the differences in the studied variables between soils collected under *Q. suber* trees with different health status. When an important reduction in soil water availability occurs, it can decrease the movement of nutrients in the soil, reducing their availability for roots and microbes (Bradford & Hsiao, 1982; Stark & Firestone, 1995). Less water and nutrient availability may reduce microbial activity and therefore, microbial respiration and organic matter mineralization (Gerten *et al.*, 2008; Knapp *et al.*, 2008), independently of the pathogen-driven oak decline. In a context of predicted longer and extremely dry periods in Mediterranean regions (Beniston *et al.*, 2007; IPCC, 2007), our results suggest that a reduction in microbial functioning and nutrient turnover can be expected, which might translate into a general loss of ecosystem functions and services.

Our models detected mainly a positive effect of the predicted warming on soil respiration and NH_4^+ availability. Warming is expected to promote microbial activity and the decomposition of soil organic matter (MacDonald *et al.*, 1995). As a result, higher soil respiration and N mineralization rates might be expected and, potentially, more inorganic nutrients could be released into the soil (Haugwitz *et al.*, 2014). However, when we detected an effect of temperature, the magnitude of this effect on soil properties was much lower than the effect of soil moisture. These results are in line with previous work conducted in Mediterranean systems that has highlighted soil moisture, but not temperature, as the limiting factor of soil processes in a context of reduced soil water content (Rey *et al.*, 2002; Emmett *et al.*, 2004). We suggest that the large magnitude of the negative impact of drought on the C and N cycles might obscure the positive effect of temperature. Therefore, we could expect a decrease in both soil respiration and N availability along with climate change in Mediterranean forests, leading to more tight C and N cycles.

In general, we detected negative additive effects of pathogen-driven mortality and climate change on P availability, which is frequently considered the main limiting nutrient in Mediterranean ecosystems (e.g. Sardans *et al.*, 2004; Morillas *et al.*, 2012). Our models showed a negative effect of pathogen-driven mortality on P availability, at least in terms of labile P after incubations. This result is in agreement with previous field studies where reductions in P were detected in areas affected by *P. cinnamomi* (Shearer *et al.*, 2009; Ávila *et al.*, 2016). This reduction may be explained by a decrease in the root activity in

soils under defoliated and dead trees, due to the importance of root exudates in regulating P availability (Schneider *et al.*, 2001). The decrease in tree root activity might have exerted a legacy effect in the soils under affected trees that might lead to lower P content (Ávila *et al.*, 2016). Interestingly, we found an interactive effect of warming and drought on P availability, consistently in both forest types. Warming led to a decrease in P availability, but that effect diminished (in closed forest) or disappeared (in open woodland) with decreasing soil moisture, suggesting an antagonistic interaction. Warming has been shown to increase microbial activity and P immobilization, decreasing its availability for plants (Sardans *et al.*, 2006; Van Meeteren *et al.*, 2007). Drought, on the contrary, usually decreases microbial activity and therefore P sequestration in microbial biomass (Criquet *et al.*, 2004; Matías *et al.*, 2011), and can even cause a release of P derived from microbial cell lysis after drought stress (Sparling *et al.*, 1985; Van Gestel *et al.*, 1993). However, this P released under drought conditions might not be accessible for plants due to water limitation of nutrient diffusion, leading to an overall negative effect of climate change on P availability. Previous studies in Mediterranean systems have found that P limits tree growth, seedling performance or microbial biomass (Sheriff *et al.*, 1986; Gallardo & Schlesinger, 1994; Gómez-Aparicio *et al.*, 2008). Therefore, a decrease in P availability as a consequence of global change would limit even further demographic and ecosystem processes in Mediterranean forests affected by pathogen-driven mortality.

Resistance of soil respiration and nutrient availability to predicted future scenarios

We found that in a context of pathogen-driven mortality, soils under affected trees had lower resistance to the warming and drought scenarios (predicted for 2050 and 2100) than soils under healthy trees. The higher resistance index found in non-affected soils may be a consequence of the high stability of soils whose microorganisms could be adapted to the particular conditions of Mediterranean systems (Curiel Yuste *et al.*, 2014). These systems are characterized by strong seasonal shifts in soil water availability as well as by high temperatures and extreme drought during summer, which might act as strong forces selecting communities dominated by drought-tolerant phenotypes (Barnard *et al.*, 2013; Curiel Yuste *et al.*, 2014). However, the process of *P. cinnamomi*-driven mortality could alter the abundance, diversity and/or functioning of the microbial soil community, as found by other studies conducted in forests affected by pest- or pathogen-induced mortality. For example, Stursova *et al.* (2014) found an increase in bacterial/fungal

biomass ratio and changes in diversity of soil microbial community in coniferous forests affected by insect-induced mortality, and Cai *et al.* (2010) detected a decrease in soil bacterial functional diversity in plots affected by Eucalypt decline. The alteration of the soil microbiota may modify the resistance of soils to future climate conditions. Our results suggest that pathogen-driven tree mortality may contribute to the alteration of biogeochemical cycles in Mediterranean ecosystems, making their soils less resistant to the predicted warming and drought under the ongoing climatic change.

Indirect effects of pathogen-tree mortality, warming and drought on soil variables

P. cinnamomi-induced tree mortality can increase relative abundance of shrubs in woody community of invaded forests (Costa *et al.*, 2011; Ibáñez *et al.*, 2017), which might greatly impact soil functioning in the following decades. Most of our models showed interactive effects between soil origin (healthy *Q. suber* trees vs. shrubs) and the predicted reduction in soil moisture. For instance, we found that under certain circumstances predicted drought resulted into lower N and P availability under pioneer shrubs than under *Q. suber* trees. A previous climate change experiment conducted in Mediterranean forests also found soils under shrubs to show different response to predicted drought than soils under trees (Matías *et al.*, 2012). Differences in terms of litter quality and production between trees and shrubs might lead to different soil characteristics such as soil fertility, which might modify the response to climate change of these soils (Rolo *et al.*, 2012; Gómez-Rey *et al.*, 2013). Our findings suggest that if the process of tree mortality leads to shrub encroachment as foreseen by previous works, climate change impacts on soil functioning might be fundamentally different compared to healthy forests, being characterized in many situations by a persistent reduction in soil respiration and nutrient availability.

Our findings clearly suggest that pathogen-induced mortality and climate change might alter biogeochemical cycles in affected forests to a larger extent than predicted when considered separately. However, the results obtained also points towards a high degree of context-dependency in the exact direction and magnitude of these changes, in line with previous studies that have reported context-dependent impacts of global change drivers (e.g. Butenschoen *et al.*, 2011; Parmesan *et al.*, 2013). For instance, although we generally found a negative interacting effect of tree mortality and predicted drought on soil respiration and N availability, positive effects were also found under some specific combinations of soil moisture and forest type. These findings clearly exemplify the

complexity inherent to predicting interactive effects among global change drivers, and support the need for further studies that allow separating main trends from context-specific idiosyncrasies.

Concluding remarks

This study represents a first experimental approach for a better understanding of the effects of predicted climate change on soil processes in Mediterranean forests affected by pathogen-induced tree mortality. Most of our models showed interactive effects between *P. cinnamomi*-induced tree mortality and drought on soil respiration and N availability. Moreover, we detected lower resistance to the warming and drought scenarios of soils under dead trees than under healthy trees. These findings clearly suggest that pathogen-induced mortality and climate change might alter biogeochemical cycles in affected forests to a much larger extent than predicted when considered separately. A previous field study conducted in the same study sites showed a decrease in soil respiration and nutrient availability under *Q. suber* trees affected by *P. cinnamomi* under current climatic conditions (Ávila *et al.*, 2016). The experimental results shown here suggest that in many cases such decrease might be exacerbated under future climatic conditions. If that is the case, the decrease in soil respiration and nutrient availability might lead to changes in ecosystem processes such as leaf litter quality and litter accumulation (Quétier *et al.*, 2007), carbon sequestration capacity (Oren *et al.*, 2001), plant mycorrhizal colonization (Smith & Read, 2008) and plant soil feedbacks (Bennett *et al.*, 2017), which will determine the future forest structure and functioning. Specifically, our results provide strong empirical support for interactions among global change stressors, in agreement with the conclusions of recent meta-analyses (Dieleman *et al.*, 2012; Zhou *et al.*, 2016). Because pest and pathogen-induced mortality is increasingly a concern in forests worldwide (Garbelotto & Pautasso, 2012; Boyd *et al.*, 2013), further research on its ecosystem impacts and interaction with other global change-stressors is very much needed for a better understanding of ecosystem functioning in a changing world.

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References

Acácio V, Holmgren M, Jansen PA, Schrotter O (2007) Multiple Recruitment Limitation Causes Arrested Succession in Mediterranean Cork Oak Systems. Ecosystems 10(7): 1220-1230

Agehara S, Warncke DD (2005) Soil moisture and temperature effects on nitrogen release from organic nitrogen sources. Soil Sci. Soc. Am. J. 69(6): 1844-1855

Allen SE, Grimshaw HM, Rowland AP (1986) Chemical analysis. In: Moore PD & Chapman SB (eds) Methods in Plant Ecology. Blackwell Scientific Publications, Oxford. p 285-344

Anderegg WR, Schwalm C, Biondi F, Camarero JJ, Koch G, Litvak M, Ogle K, Shaw JD, Shevliakova E, Williams A (2015) Pervasive drought legacies in forest ecosystems and their implications for carbon cycle models. Science 349(6247): 528-532

Auyeung DSN, Suseela V, Dukes JS (2013) Warming and drought reduce temperature sensitivity of nitrogen transformations. Global Change Biol. 19(2): 662-676

Ávila JM, Gallardo A, Ibáñez B, Gómez-Aparicio L (2016) Quercus suber dieback alters soil respiration and nutrient availability in Mediterranean forests. J. Ecol. 104(5): 1441-1452

Ávila JM, Linares JC, García-Nogales A, Sánchez ME, Gómez-Aparicio L (2017) Across-scale patterning of plant–soil–pathogen interactions in Quercus suber decline. European Journal of Forest Research 136(4): 677-688

Bai E, Li S, Xu W, Li W, Dai W, Jiang P (2013) A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. New Phytol. 199(2): 431-440

Barnard RL, Osborne CA, Firestone MK (2013) Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J 7(11): 2229-2241

Beniston M, Stephenson DB, Christensen OB, Ferro CAT, Frei C, Goyette S, Halsnaes K, Holt T, Jylhä K, Koffi B, Palutikof J, Schöll R, Semmler T, Woth K (2007) Future extreme events in European climate: an exploration of regional climate model projections. Clim. Change 81(1): 71-95

Bennett JA, Maherali H, Reinhart KO, Lekberg Y, Hart MM, Klironomos J (2017) Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. Science 355(6321): 181-184

Boyd I, Freer-Smith P, Gilligan C, Godfray H (2013) The consequence of tree pests and diseases for ecosystem services. Science 342(6160): 1235773

Bradford KJ, Hsiao TC (1982) Physiological Responses to Moderate Water Stress. In: Lange OL, Nobel PS, Osmond CB & Ziegler H (eds) Physiological Plant Ecology II. Encyclopedia of Plant Physiology. Springer Berlin Heidelberg. p 263-324

Brasier CM (1992) Oak tree mortality in Iberia. Nature 360(6404): 539

Burgess TI, Scott JK, Mcdougall KL, Stukely MJC, Crane C, Dunstan WA, Brigg F, Andjic V, White D, Rudman T, Arentz F, Ota N, Hardy GESJ (2017) Current and projected global distribution of Phytophthora cinnamomi, one of the world's worst plant pathogens. Global Change Biol. 23(4): 1661-1674

Burnham KP, Anderson DR (2002) Model Selection and Multi-Model Inference : A Practical Information-Theoretic Approach. Springer, Secaucus, NJ, USA

Butenschoen O, Scheu S, Eisenhauer N (2011) Interactive effects of warming, soil humidity and plant diversity on litter decomposition and microbial activity. Soil Biology & Biochemistry 43(9): 1902-1907

Cai YF, Barber P, Dell B, O'Brien P, Williams N, Bowen B, Hardy G (2010) Soil bacterial functional diversity is associated with the decline of Eucalyptus gomphocephala. For. Ecol. Manage. 260(6): 1047-1057

Camilo-Alves CSP, da Clara MIE, Ribeiro NA (2013) Decline of Mediterranean oak trees and its association with Phytophthora cinnamomi: a review. European Journal of Forest Research 132(3): 411-432

Cao MK, Woodward FI (1998) Dynamic responses of terrestrial ecosystem carbon cycling to global climate change. Nature 393(6682): 249-252

Cobb RC, Eviner VT, Rizzo DM (2013) Mortality and community changes drive sudden oak death impacts on litterfall and soil nitrogen cycling. New Phytol. 200(2): 422-431

Costa A, Madeira M, Lima Santos J, Oliveira Â (2011) Change and dynamics in Mediterranean evergreen oak woodlands landscapes of Southwestern Iberian Peninsula. Landscape Urban Plann. 102(3): 164-176

Cox NJ (2011) Stata tip 96: Cube roots. Stata Journal 11(1): 149

Crawley MJ (2007) The R book. Wiley, UK

Criquet S, Ferre E, Farnet AM, Le petit J (2004) Annual dynamics of phosphatase activities in an evergreen oak litter: influence of biotic and abiotic factors. Soil Biology and Biochemistry 36(7): 1111-1118

Curiel Yuste J, Fernandez-Gonzalez A, Fernandez-Lopez M, Ogaya R, Peñuelas J, Sardans J, Lloret F (2014) Strong functional stability of soil microbial communities under semiarid Mediterranean conditions and subjected to long-term shifts in baseline precipitation. Soil Biology and Biochemistry 69: 223-233

Cherubini P, Fontana G, Rigling D, Dobbertin M, Brang P, Innes JL (2002) Tree-life history prior to death: two fungal root pathogens affect tree-ring growth differently. J. Ecol. 90(5): 839-850

Dieleman WIJ, Vicca S, Dijkstra FA, Hagedorn F, Hovenden MJ, Larsen KS, Morgan JA, Volder A, Beier C, Dukes JS, King J, Leuzinger S, Linder S, Luo Y, Oren R, De Angelis P, Tingey D, Hoosbeek MR, Janssens IA (2012) Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO2 and temperature. Global Change Biol. 18(9): 2681-2693

Dijkstra FA, Pendall E, Morgan JA, Blumenthal DM, Carrillo Y, LeCain DR, Follett RF, Williams DG (2012) Climate change alters stoichiometry of phosphorus and nitrogen in a semiarid grassland. New Phytol. 196(3): 807-815

Durán J, Delgado-Baquerizo M, Rodríguez A, Covelo F, Gallardo A (2013) Ionic exchange membranes (IEMs): A good indicator of soil inorganic N production. Soil Biology & Biochemistry 57: 964-968

Emmett BA, Beier C, Estiarte M, Tietema A, Kristensen HL, Williams D, Peñuelas J, Schmidt I, Sowerby A (2004) The response of soil processes to climate change: Results from manipulation studies of shrublands across an environmental gradient. Ecosystems 7(6): 625-637

Franzluebbers AJ (1999) Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biology and Biochemistry 31(8): 1083-1090

Gallardo A, Schlesinger WH (1994) Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biology and Biochemistry 26(10): 1409-1415

Gandhi KJK, Herms DA (2010) Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. Biol. Invasions 12(2): 389-405

Garbelotto M, Pautasso M (2012) Impacts of exotic forest pathogens on Mediterranean ecosystems: four case studies. Eur. J. Plant Pathol. 133(1): 101-116

García-Palacios P, Milla R, Alvaro-Sanchez M, Martín-Robles N, Maestro M (2013) Application of a high-throughput laboratory method to assess litter decomposition rates in multiple-species experiments. Soil Biology & Biochemistry 57: 929-932

García C, Hernández T, Roldán A, Martín A (2002) Effect of plant cover decline on chemical and microbiological parameters under Mediterranean climate. Soil Biology & Biochemistry 34(5): 635-642

Gerten D, Luo Y, Le Maire G, Parton WJ, Keough C, Weng E, Beier C, Ciais P, Cramer W, Dukes JS, Hanson PJ, Knapp AAK, Linder S, Nepstad D, Rustad L, Sowerby A (2008) Modelled effects of precipitation on ecosystem carbon and water dynamics in different climatic zones. Global Change Biol. 14(10): 2365-2379

Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. Global Planet. Change 63(2–3): 90-104

Gómez-Aparicio L, Ibáñez B, Serrano MS, De Vita P, Ávila JM, Pérez-Ramos IM, García LV, Sánchez ME, Marañón T (2012) Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration. New Phytol. 194(4): 1014-1024

Gómez-Aparicio L, Pérez-Ramos IM, Mendoza I, Matías L, Quero JL, Castro J, Zamora R, Marañón T (2008) Oak seedling survival and growth along resource gradients in Mediterranean forests: Implications for regeneration in current and future environmental scenarios. Oikos 117(11): 1683-1699

Gómez-Rey MX, Madeira M, Gonzalez-Prieto SJ, Coutinho J (2013) Soil C and N dynamics in a Mediterranean oak woodland with shrub encroachment. Plant Soil 371(1): 339-354

Grimm NB, Chapin FS, Bierwagen B, Gonzalez P, Groffman PM, Luo Y, Melton F, Nadelhoffer K, Pairis A, Raymond PA, Schimel J, Williamson CE (2013) The impacts of climate change on ecosystem structure and function. Front. Ecol. Environ. 11(9): 474-482

Haugwitz MS, Bergmark L, Prieme A, Christensen S, Beier C, Michelsen A (2014) Soil microorganisms respond to five years of climate change manipulations and

elevated atmospheric CO2 in a temperate heath ecosystem. Plant Soil 374(1-2): 211-222

Heisler JL, Weltzin JF (2006) Variability matters: towards a perspective on the influence of precipitation on terrestrial ecosystems. New Phytol. 172(2): 189-192

Hicke JA, Allen CD, Desai AR, Dietze MC, Hall RJ, Hogg EH, Kashian DM, Moore D, Raffa KF, Sturrock RN, Vogelmann J (2012) Effects of biotic disturbances on forest carbon cycling in the United States and Canada. Global Change Biol. 18(1): 7-34

Ibáñez B, Gómez-Aparicio L, Ávila JM, Pérez-Ramos IM, Marañón T (2017) Effects of Quercus suber Decline on Woody Plant Regeneration: Potential Implications for Successional Dynamics in Mediterranean Forests. Ecosystems 20(3): 630-644

IPCC (2007) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: The Core Writing Team (ed). Cambridge University Press, IPCC, Geneva, Switzerland.

IPCC (2014a) Climate Change 2014: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In., Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

IPCC (2014b) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: The Core Writing Team, Pachauri RK & Meyer L (eds). IPCC, Geneva, Switzerland.

Knapp AK, Beier C, Briske DD, Classen AT, Luo Y, Reichstein M, Smith MD, Smith SD, Bell JE, Fay PA, Heisler JL, Leavitt SW, Sherry R, Smith B, Weng E (2008) Consequences of More Extreme Precipitation Regimes for Terrestrial Ecosystems. Bioscience 58(9): 811-821

Kouno K, Tuchiya Y, Ando T (1995) Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biology and Biochemistry 27(10): 1353-1357

Loo JA (2009) Ecological impacts of non-indigenous invasive fungi as forest pathogens. Biol. Invasions 11(1): 81-96

Lovett GM, Arthur MA, Weathers KC, Griffin JM (2010) Long-term changes in forest carbon and nitrogen cycling caused by an introduced pest/pathogen complex. Ecosystems 13(8): 1188-1200

Lovett GM, Canham CD, Arthur MA, Weathers KC, Fitzhugh RD (2006) Forest ecosystem responses to exotic pests and pathogens in eastern North America. Bioscience 56(5): 395-405

Luo Y, Gerten D, Le Maire G, Parton WJ, Weng E, Zhou X, Keough C, Beier C, Ciais P, Cramer W, Dukes JS, Emmett B, Hanson PJ, Knapp A, Linder S, Nepstad DAN, Rustad L (2008) Modeled interactive effects of precipitation, temperature, and [CO2] on ecosystem carbon and water dynamics in different climatic zones. Global Change Biol. 14(9): 1986-1999

Lloret F, Mattana S, Curiel Yuste J (2015) Climate-induced die-off affects plant–soil–microbe ecological relationship and functioning. FEMS Microbiol. Ecol. 91(2): 1-12

MacDonald NW, Zak DR, Pregitzer KS (1995) Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralization. Soil Sci. Soc. Am. J. 59(1): 233-240

Manabe S, Milly PCD, Wetherald R (2004) Simulated long-term changes in river discharge and soil moisture due to global warming. Hydrological Sciences Journal-Journal Des Sciences Hydrologiques 49(4): 625-642

Matías L, Castro J, Zamora R (2011) Soil-nutrient availability under a global-change scenario in a Mediterranean mountain ecosystem. Global Change Biol. 17(4): 1646-1657

Matías L, Castro J, Zamora R (2012) Effect of simulated climate change on soil respiration in a Mediterranean-type ecosystem: rainfall and habitat type are more important than temperature or the soil carbon pool. Ecosystems 15(2): 299-310

McCullagh P, Nelder JA (1989) Generalized Linear Models, Second Edition. Taylor & Francis,

Milla R, Cornelissen JH, van Logtestijn RS, Toet S, Aerts R (2006) Vascular plant responses to elevated CO2 in a temperate lowland Sphagnum peatland. Plant Ecol. 182(1-2): 13-24

Moreno G, Obrador JJ (2007) Effects of trees and understorey management on soil fertility and nutritional status of holm oaks in Spanish dehesas. Nutrient Cycling in Agroecosystems 78(3): 253-264

Morillas L, Duran J, Rodriguez A, Roales J, Gallardo A, Lovett GM, Groffman PM (2015) Nitrogen supply modulates the effect of changes in drying-rewetting frequency on soil C and N cycling and greenhouse gas exchange. Global Change Biol. 21(10): 3854-3863

Morillas L, Gallardo A, Portillo-Estrada M, Covelo F (2012) Nutritional status of Quercus suber populations under contrasting tree dieback. Forestry 85(3): 369-378

Nakicenovic N, Swart R (2000) Special report on emissions scenarios. Cambridge University Press, Cambridge, UK

Ninyerola M, Pons X, Roure JM (2005) Atlas climático digital de la Península Ibérica: metodología y aplicaciones en bioclimatología y geobotánica. Universidad Autónoma de Barcelona, Barcelona

Ojeda F, Marañón T, Arroyo J (2000) Plant diversity patterns in the Aljibe Mountains (S. Spain): A comprehensive account. Biodivers. Conserv. 9(9): 1323-1343

Oren R, Ellsworth DS, Johnsen KH, Phillips N, Ewers BE, Maier C, Schafer KVR, McCarthy H, Hendrey G, McNulty SG, Katul GG (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO2-enriched atmosphere. Nature 411(6836): 469-472

Orwin KH, Wardle DA (2004) New indices for quantifying the resistance and resilience of soil biota to exogenous disturbances. Soil Biology and Biochemistry 36(11): 1907-1912

Paine RT, Tegner MJ, Johnson EA (1998) Compounded perturbations yield ecological surprises. Ecosystems 1(6): 535-545

Parmesan C, Burrows MT, Duarte CM, Poloczanska ES, Richardson AJ, Schoeman DS, Singer MC (2013) Beyond climate change attribution in conservation and ecological research. Ecol. Lett. 16(s1): 58-71

Peñuelas J, Filella I, Comas P (2002) Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. Global Change Biol. 8(6): 531-544

Petchey OL, McPhearson PT, Casey TM, Morin PJ (1999) Environmental warming alters food-web structure and ecosystem function. Nature 402: 69

Piggott JJ, Townsend CR, Matthaei CD (2015) Reconceptualizing synergism and antagonism among multiple stressors. Ecology and Evolution 5(7): 1538-1547

Qian P, Schoenau JJ (2002) Practical applications of ion exchange resins in agricultural and environmental soil research. Can. J. Soil Sci. 82(1): 9-21

Quétier F, Thébault A, Lavorel S (2007) Plant traits in a state and transition framework as markers of ecosystem response to land-use change. Ecol. Monogr. 77(1): 33-52

R Core Team (2014) R: A language and environment for statistical computing. In. R Foundation for Statistical Computing, Vienna, Austria.

Reinhart KO, Rinella MJ (2016) A common soil handling technique can generate incorrect estimates of soil biota effects on plants. New Phytol. 210(3): 786-789

Rey A, Pegoraro E, Tedeschi V, De Parri I, Jarvis PG, Valentini R (2002) Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. Global Change Biol. 8(9): 851-866

Rolo V, López-Díaz ML, Moreno G (2012) Shrubs affect soil nutrients availability with contrasting consequences for pasture understory and tree overstory production and nutrient status in Mediterranean grazed open woodlands. Nutrient Cycling in Agroecosystems 93(1): 89-102

Sánchez ME, Caetano P, Ferraz J, Trapero A (2002) Phytophthora disease of Quercus ilex in south-western Spain. For. Pathol. 32(1): 5-18

Sardans J, Peñuelas J, Estiarte M (2006) Warming and drought alter soil phosphatase activity and soil P availability in a Mediterranean shrubland. Plant Soil 289(1-2): 227-238

Sardans J, Rodà F, Peñuelas J (2004) Phosphorus limitation and competitive capacities of Pinus halepensis and Quercus ilex subsp. rotundifolia on different soils. Plant Ecol. 174(2): 305-317

Schneider K, Turrión MB, Grierson PF, Gallardo JF (2001) Phosphatase activity, microbial phosphorus, and fine root growth in forest soils in the Sierra de Gata, western central Spain. Biol. Fertility Soils 34(3): 151-155

Schoenau JJ, Huang WZ (1991) Anion-exchange membrane, water, and sodium-bicarbonate extractions as soil tests for phosphorus. Commun. Soil Sci. Plant Anal. 22(5-6): 465-492

Shearer BL, Crane CE, Fairman RG, Dunne CP (2009) Ecosystem dynamics altered by pathogen-mediated changes following invasion of Banksia woodland and Eucalyptus marginata forest biomes of south-western Australia by Phytophthora cinnamomi. Austral. Plant Pathol. 38(4): 417-436

Sheriff D, Nambiar E, Fife D (1986) Relationships between nutrient status, carbon assimilation and water use efficiency in Pinus radiata (D. Don) needles. Tree Physiology 2(1-2-3): 73-88

Sims GK, Ellsworth TR, Mulvaney RL (1995) Microscale determination of inorganic nitrogen in water and soil extracts. Commun. Soil Sci. Plant Anal. 26(1-2): 303-316

Smith SE, Read DJ (2008) Mycorrhizal Symbiosis. Academic Press, UK

Sparling GP, Whale KN, Ramsay AJ (1985) Quantifying the contribution from the soil microbial biomass to the extractable P-levels of fresh and air-dried soils. Aust. J. Soil Res. 23(4): 613-621

Stark JM, Firestone MK (1995) Mechanisms for soil-moisture effects on activity of nitrifying bacteria. Appl. Environ. Microbiol. 61(1): 218-221

Stursova M, Snajdr J, Cajthaml T, Barta J, Santruckova H, Baldrian P (2014) When the forest dies: the response of forest soil fungi to a bark beetle-induced tree dieback. Isme Journal 8(9): 1920-1931

Subler S, Blair JM, Edwards CA (1995) Using anion-exchange membranes to measure soil nitrate availability and net nitrification. Soil Biology & Biochemistry 27(7): 911-917

Trumbore S, Brando P, Hartmann H (2015) Forest health and global change. Science 349(6250): 814-818

Van Gestel M, Merckx R, Vlassak K (1993) Microbial biomass responses to soil drying and rewetting-the fate of fast-growing and slow-growing microorganisms in soils from different climates. Soil Biology & Biochemistry 25(1): 109-123

Van Meeteren MJM, Tietema A, Westerveld JW (2007) Regulation of microbial carbon, nitrogen, and phosphorus transformations by temperature and moisture during decomposition of Calluna vulgaris litter. Biol. Fertility Soils 44(1): 103-112

Vicente-Serrano SM, Lopez-Moreno JI, Beguería S, Lorenzo-Lacruz J, Sanchez-Lorenzo A, García-Ruiz JM, Azorin-Molina C, Morán-Tejeda E, Revuelto J, Trigo R, Coelho F, Espejo F (2014) Evidence of increasing drought severity caused by temperature rise in southern Europe. Environmental Research Letters 9(4): 044001

Vitousek PM (1994) Beyond global warming: Ecology and global change. Ecology 75(7): 1861-1876

Wang X, Comita LS, Hao Z, Davies SJ, Ye J, Lin F, Yuan Z (2012) Local-scale drivers of tree survival in a temperate forest. PLoS One 7(2): e29469

Wang XL, Zwiers FW, Swail VR (2004) North Atlantic Ocean Wave Climate Change Scenarios for the Twenty-First Century. J. Clim. 17(12): 2368-2383

Wetherald RT, Manabe S (2002) Simulation of hydrologic changes associated with global warming. Journal of Geophysical Research-Atmospheres 107(D19): 4379

Zhou L, Zhou X, Shao J, Nie Y, He Y, Jiang L, Wu Z, Hosseini Bai S (2016) Interactive effects of global change factors on soil respiration and its components: a meta-analysis. Global Change Biol. 22(9): 3157-3169

Ziadi N, Simard RR, Allard G, Lafond J (1999) Field evaluation of anion exchange membranes as a N soil testing method for grasslands. Can. J. Soil Sci. 79(2): 281-294

Supporting information

Figure S1 Location map of Los Alcornocales Natural Park. Pictures and The number of soil samples are shown in brackets.

Sampling: Soil origins

Open Woodland

- Healthy *Quercus suber* trees (5)
- Defoliated *Q. suber* trees (5)
- Dead *Q. suber* trees (5)
- Late-successional shrub: *Pistacia lentiscus* (5)
- Pioneer shrub: *Teline linifolia (5)*

Closed forest

- Healthy *Q. suber* trees (5)
- Defoliated *Q. suber* trees (5)
- Dead *Q. suber* trees (5)
- Late-successional shrub: *Erica arborea* (5)
- Pioneer shrub: *Ulex borgiae (5)*

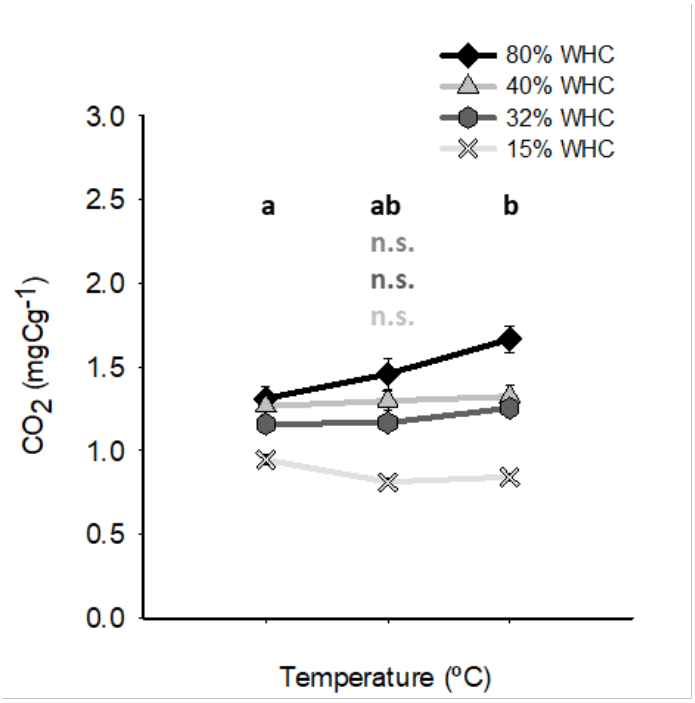

Figure S2 Soil respiration (mean ± SE) for each soil temperature at each soil moisture level (n=15) in closed forest. The lower case indicates significant differences in soil respiration between temperature levels within moisture levels ($p<0.05$).

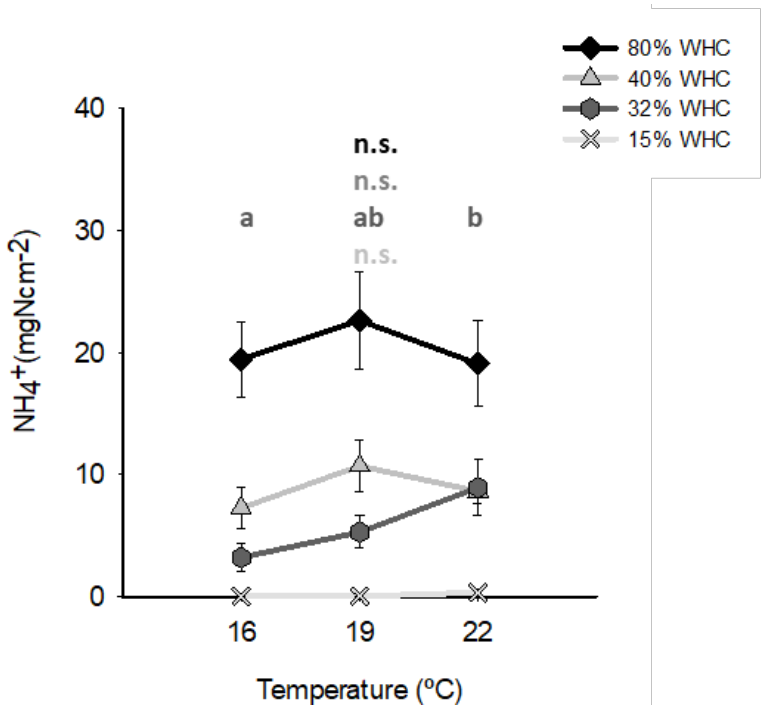

Figure S3 NH_4^+ availability (mean ± SE) for each soil temperature at each soil moisture level (n=15) in closed forest. The lower case indicates significant differences in NH_4^+ availability between temperature levels within moisture levels ($p<0.05$).

DISCUSIÓN GENERAL

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En las últimas décadas se ha incrementado el interés sobre el fenómeno del aumento de la mortalidad arbórea en bosques de todo el mundo (e.g. van Mantgem *et al.*, 2009; Allen *et al.*, 2010; Carnicer *et al.*, 2011; Trumbore *et al.*, 2015). A pesar del elevado potencial que tiene este incremento de la mortalidad para producir alteraciones en el funcionamiento del ecosistema (p. ej. Anderegg *et al.*, 2013), la información que existe a día de hoy es limitada y está sesgada a determinados eventos de mortalidad asociados al cambio climático y las plagas de insectos (ver revisiones de Hicke *et al.*, 2012; Wang *et al.*, 2012; Anderegg *et al.*, 2015). Para mejorar nuestro conocimiento sobre el efecto del aumento de los procesos de decaimiento y mortalidad arbórea sobre el ecosistema, esta tesis doctoral se ha centrado en el estudio de las consecuencias que tienen cambios en la estructura y composición de bosques mixtos mediterráneos afectados por decaimiento sobre los principales ciclos biogeoquímicos (carbono, nitrógeno y fósforo) y la comunidad microbiana, así como su interacción con el cambio climático. Concretamente el estudio se ha realizado en bosques mixtos de *Quercus suber* situados en el sur de la península ibérica y afectados por decaimiento asociado al patógeno invasor *Phytophthora cinnamomi*. Esta tesis doctoral representa un novedoso intento de conectar comunidades de plantas complejas en las que se entremezclan árboles y matorrales de diferentes especies y con distinto estado de salud, con la variabilidad espacial y temporal de procesos y características del suelo. Los resultados de esta tesis doctoral contribuyen a mejorar el entendimiento del funcionamiento del ecosistema en un contexto de cambio global, permitiendo realizar predicciones más precisas sobre las alteraciones que se producirán en el suelo ante diferentes escenarios de decaimiento forestal y cambio climático.

Vulnerabilidad del alcornocal ante el decaimiento asociado al patógeno *P. cinnamomi*

En el capítulo 2 de la tesis doctoral se hipotetizó que individuos de *Q. suber* afectados por decaimiento mostraran una respuesta fisiológica, en términos de eficiencia del uso del agua y crecimiento secundario, como se ha observado en otros fenómenos de decaimiento de otras especies arbóreas (Linares y Camarero, 2012; Sangüesa-Barreda *et al.*, 2013). *P. cinnamomi* provoca una reducción en la abundancia de raíces finas en árboles infectados (Corcobado *et al.*, 2013), lo cual podría afectar a la conductancia

estomática, incrementándose la eficiencia del uso del agua. De hecho, se ha observado que plántulas de especies de *Quercus* afectadas por el patógeno *P. cinnamomi* muestran una mayor eficiencia del uso del agua que les conferiría una mayor tolerancia al estrés hídrico (Luque *et al.*, 1999; Maurel *et al.*, 2001; Robin *et al.*, 2001; Sghaier-Hammami *et al.*, 2013). Aunque en el capítulo 2 se detectó una relación positiva entre la abundancia del patógeno *P. cinnamomi* en el suelo y la eficiencia en el uso del agua de los individuos de *Q. suber*, este efecto no se tradujo en un patrón de incremento de la eficiencia en el uso del agua de árboles defoliados respecto a árboles sano. Este resultado sugiere un desacople entre los síntomas de defoliación del árbol y su respuesta fisiológica. Asimismo, la falta de una clara relación entre el estado de salud y la eficiencia del uso del agua y el crecimiento secundario parecen demostrar una gran vulnerabilidad de *Q. suber* al ataque del patógeno *P. cinnamomi*. Los árboles infectados no parecen poder adaptar su fisiología a la pérdida de raíces finas, de manera que su respuesta al estrés hídrico resultante sería la defoliación y eventual muerte del individuo.

La invasión reciente de *P. cinnamomi* y por tanto la falta de co-evolución entre el patógeno invasor y el hospedador podría ser la causa de la elevada vulnerabilidad mostrada por los individuos de *Q. suber* a este patógeno. Esta elevada vulnerabilidad se traduce en un descenso en el vigor e incremento de la mortalidad de esta especie y de los bosques que forman. Más de la mitad de los individuos de *Q. suber* mostraban signos de decaimiento (defoliación o muerte) en la mayoría de las parcelas estudiadas, alcanzando en algunos casos porcentajes por encima del 70%. Aunque ciertas especies de matorral mediterráneo pueden actuar como hospedadores del patógeno (Moreira y Martins, 2005), su susceptibilidad es mucho menor que la de *Q. suber*, lo que puede explicar que no se hayan detectado daños en la parte aérea de otras especies del sotobosque. Por ello, el decaimiento asociado a *P. cinnamomi* está afectando y afectará en un futuro a la abundancia relativa de las especies coexistentes en estos bosques y, por tanto, a su estructura. La elevada incidencia de *P. cinnamomi* podría alterar las relaciones planta-suelo en los bosques mixtos de *Q. suber*, lo que podría tener consecuencias para el funcionamiento del ecosistema, como se ha mostrado en los capítulos siguientes de esta tesis.

Consecuencias del decaimiento de *Q. suber* en el funcionamiento del ecosistema

En el transcurso de esta tesis se han mostrado evidencias de cómo la defoliación y muerte de individuos de *Q. suber* asociada al patógeno invasor *P. cinnamomi* provocan importantes cambios en variables clave de los ciclos del carbono, nitrógeno y fósforo, sin alterar la diversidad ni la abundancia de las comunidades microbianas (capítulos 3 y 4). En estos trabajos se han conectado por primera vez vecindades complejas, en las que se entremezclan árboles de diferentes especies, tamaños y estados de salud, con la variabilidad espaciotemporal de procesos del suelo. La aproximación de los modelos de vecindad utilizada en estos trabajos ha permitido la estimación de los efectos per cápita de los árboles sobre variables clave de los ciclos biogeoquímicos y de la comunidad microbiana en bosques mixtos mediterráneos afectados por decaimiento. Esta información resulta clave para poder entender y predecir los efectos del decaimiento asociado a un patógeno invasor en el funcionamiento del ecosistema.

Efectos directos del decaimiento de Q. suber en los ciclos biogeoquímicos y la comunidad microbiana

La infección por *P. cinnamomi* puede alterar las relaciones planta-suelo, lo que puede tener consecuencias importantes para el procesado de la materia orgánica y el ciclo del carbono en bosques afectados por este patógeno. Los resultados del capítulo 3 apoyan la hipótesis planteada, en la que se esperaban reducciones en la respiración del suelo bajo árboles en decaimiento. El descenso de la productividad de los árboles podría ser responsable del descenso de la respiración del suelo debido, fundamentalmente, al descenso en la respiración de raíces y la rizosfera asociada (Nuckolls *et al.*, 2009; Edburg *et al.*, 2011; Flower *et al.*, 2013). Los resultados de esta tesis apoyan los hallazgos de trabajos previos que simulan el ataque de patógenos en sistemas naturales (Högberg y Read, 2006; Nuckolls *et al.*, 2009; Levy-Varon *et al.*, 2012; Levy-Varon *et al.*, 2014). Además, los resultados del capítulo 4 muestran cómo no solo la respiración autótrofa puede verse afectada por el decaimiento de *Q. suber*, sino que incluso la respiración microbiana es menor bajo árboles defoliados y muertos, al menos bajo condiciones óptimas en el laboratorio.

El decaimiento de *Q. suber* también provocó cambios en compuestos clave del ciclo del carbono. Sin embargo, estos efectos variaron en función del tipo de bosque estudiado.

Recientes revisiones han mostrado cómo perturbaciones asociadas al ataque de insectos y patógenos pueden provocar incrementos en la cantidad de compuestos de carbono con bajos tiempos de residencia y descensos de los compuestos de carbono con altos tiempos de residencia (Hicke *et al.*, 2012; Zhang *et al.*, 2015). En los bosques cerrados de *Q. suber* y *Quercus canariensis*, caracterizados por un bajo contenido en arcilla, se observó que el decaimiento asociado a *P. cinnamomi* provocó un descenso en el carbono total del suelo, pero un incremento en el carbono orgánico disuelto, tal y como se predijo en la segunda hipótesis del capítulo 4. Sin embargo, en los bosques abiertos de *Q. suber* y *Olea europaea,* donde el contenido de arcilla es significativamente mayor que en los bosques cerrados, se detectó un incremento en el carbono total del suelo bajo árboles en decaimiento. El papel protector de la arcilla en la descomposición de la materia orgánica (p.ej. Van Veen y Kuikman, 1990; Hassink y Whitmore, 1997; Müller y Höper, 2004) permitiría explicar la acumulación del carbono total bajo árboles defoliados y muertos en los bosques abiertos. Estos resultados parecen demostrar que información sobre las características del suelo, tal como la textura, podría ser esencial para predecir el efecto del decaimiento forestal sobre el reciclado de la materia orgánica.

El decaimiento de *Q. suber* también provocó cambios en la disponibilidad de nutrientes limitantes tales como el fósforo y el nitrógeno. Los resultados de los capítulos 3 y 5 mostraron un descenso de la disponibilidad de fósforo bajo árboles defoliados y muertos, tal y como se predijo en la hipótesis 1 del capítulo 3. En sistemas limitados por fósforo, el descenso de la actividad de las raíces podría ser el responsable de la disminución de la disponibilidad de este nutriente (Högberg *et al.*, 2001; Xiong *et al.*, 2011), debido al papel que tienen las raíces en la liberación de fósforo disponible (Schneider *et al.*, 2001). A diferencia del fósforo, el efecto sobre el nitrógeno fue mucho más complejo y estuvo fuertemente influenciado por el tipo de bosque estudiado. La defoliación y muerte de *Q. suber* provocó un incremento del nitrato, pero un descenso del amonio, posiblemente debido al incremento en las tasas de nitrificación (capítulo 4). Sin embargo, este efecto solo se detectó en los bosques abiertos, con un alto contenido en arcilla, que podría retener los compuestos de nitrógeno inorgánico (Allison, 1973; Hassink, 1997). Este elevado contenido en arcilla podría explicar el que no se hayan detectado diferencias en la disponibilidad de nitrógeno medido mediante resinas de intercambio iónico en dichos bosques (capítulo 3). Por su parte, en los bosques cerrados, donde el contenido de arcilla es mucho menor, no se detectaron diferencias en la cantidad de nitrógeno inorgánico bajo árboles en decaimiento y sanos. Esto pudo estar influenciado

por el elevado nivel de difusión del nitrógeno inorgánico en estos suelos, mientras que sí que se detectaron diferencias en la cantidad de nitrógeno disponible medido mediante resinas, con mayores valores de nitrógeno disponible bajo árboles defoliados y muertos que bajo árboles sanos, tal y como se ha encontrado en trabajos previos sobre consecuencias de incremento de la mortalidad asociado a plagas de insectos y cambio climático (e.g. Kizlinski *et al.*, 2002; Clow *et al.*, 2011; Wang *et al.*, 2012). Los resultados obtenidos en estos capítulos parecen sugerir que el decaimiento de *Q. suber* podría generar en algunos casos desacoples entre los ciclos del nitrógeno y el fósforo, lo que a su vez podría ocasionar impactos en el funcionamiento del ecosistema con consecuencias negativas en la productividad primaria y la descomposición de la materia orgánica (Finzi *et al.*, 2011).

Los cambios detectados en el funcionamiento del ecosistema debido al decaimiento provocado por *P. cinnamomi* no estuvieron acoplados a cambios en la diversidad y cantidad de la comunidad microbiana (capítulo 4). Si bien eran esperables cambios en la comunidad microbiana debido a las posibles alteraciones en la composición de las fuentes de carbono que llegan al suelo asociadas al proceso de defoliación del árbol, no se detectaron diferencias en la diversidad funcional o en la biomasa microbiana bajo árboles en decaimiento y árboles sanos. Nuestros resultados demuestran que es posible que el cambio global pueda generar alteraciones en el funcionamiento del sistema sin que se detecten cambios en la abundancia ni diversidad (al menos funcional) de la comunidad microbiana del suelo. Este resultado aporta información muy valiosa al estudio de la relación biodiversidad-funcionamiento (e.g. Allison y Martiny, 2008; Bell *et al.*, 2009; Delgado-Baquerizo *et al.*, 2016) y apoya la idea de que la relación positiva entre biodiversidad del suelo y funcionamiento ecosistémico no se puede aplicar universalmente (p. ej. Bell *et al.*, 2009).

Efectos indirectos del decaimiento de Q. suber en los ciclos biogeoquímicos y la comunidad microbiana

En los bosques mixtos afectados por decaimiento asociado a *P. cinnamomi* se ha detectado una elevada mortalidad de individuos de *Q. suber*, mientras que las especies arbóreas co-existentes (*Olea europaea, Quercus canariensis*) no parecen verse afectadas. Esto está provocando un cambio en la abundancia relativa de las especies en el dosel arbóreo (capítulo 2). Además, los huecos que dejan los árboles muertos son rápidamente ocupados por especies de matorral sucesional, que tampoco muestran signos de afectación

por el patógeno lo cual podría incrementar la abundancia de matorral en estos bosques durante las próximas décadas (Ibáñez *et al.*, 2017). Esto se debe a que las dinámicas sucesionales en estos bosques son muy lentas y el establecimiento de los *Quercus* en estos huecos es muy bajo (Ibáñez *et al.*, 2017). Por este motivo y para poder comprender el impacto del decaimiento de *Q. suber* en los bosques afectados, es esencial poder conocer sus efectos indirectos asociados a la sustitución de especies provocadas por *P. cinnamomi*. Para ello es necesario comparar el efecto de especies coexistentes no afectadas por decaimiento con el efecto de los individuos sanos de *Q. suber* sobre los procesos y características del suelo, ya que estudios previos han demostrado que el efecto indirecto de las perturbaciones bióticas del arbolado puede ser de mayor magnitud que el efecto directo provocado por la propia defoliación y muerte del árbol (Lovett *et al.*, 2010; Cobb *et al.*, 2013).

En general, se han detectado importantes diferencias en el efecto de las especies arbóreas coexistentes con *Q. suber* en la disponibilidad de nutrientes del suelo, lo que conllevaría cambios en el funcionamiento ecosistémico en caso de darse una sustitución de especies a lo largo del proceso de sucesión secundaria (capítulos 3 y 4). En este sentido, se ha observado que la posible sustitución de *Q. suber* por *Q. canariensis* se traduciría en suelos más fértiles con una mayor disponibilidad de nitrógeno, carbono orgánico disuelto y hexosas, mientras que la sustitución por *O. europea* generaría suelos más pobres con menor disponibilidad de nitrógeno y fósforo. Por su parte, los resultados del capítulo 5 nos sugieren que, si se produjera un incremento del matorral, el impacto del cambio climático en estos suelos sería diferente al que ocurriría en suelos bajo *Q. suber*, de forma que los suelos bajo matorral tenderían a mostrar menores tasas de respiración y menor disponibilidad de nutrientes que los suelos bajo *Q. suber*. En general, los resultados de estos trabajos demuestran que los efectos indirectos asociados a la mortalidad de *Q. suber* estarán fuertemente influenciados por la identidad de la especie sustituta. Por tanto, resulta fundamental incluir el estudio del efecto de especies coexistente no afectadas en bosques mixtos, para poder comprender el efecto del decaimiento en los ciclos biogeoquímicos a medio y largo plazo.

Caracterización de la "huella" del árbol en los procesos del suelo

Los modelos de vecindad permiten analizar el efecto del tamaño y la distancia a los árboles vecinos sobre las variables estudiadas gracias a los parámetros α y β

(respectivamente) del índice de vecindad (NI, ver sección Introducción general). Los resultados de los capítulos 3 y 4 mostraron una elevada variabilidad en el efecto del tamaño de los árboles vecinos sobre las variables estudiadas. Sin embargo, el elevado valor del parámetro α en la mayoría de los modelos sugiere que los árboles de mayor tamaño tienen efectos desproporcionadamente grandes sobre la respiración del suelo y la disponibilidad de nutrientes. Estos resultados están en línea con trabajos previos que han mostrado el papel de los árboles de mayor tamaño en la variación espacial de la respiración y los nutrientes del suelo (Ludwig *et al.*, 2004; Søe y Buchmann, 2005). Además del efecto del tamaño del árbol, los modelos de vecindad también permiten evaluar la forma que tiene la huella del árbol, es decir, cómo varía el efecto del árbol con la distancia (Canham y Uriarte, 2006; Gómez-Aparicio y Canham, 2008). Los resultados de esta tesis mostraron una fuerte variabilidad en la forma de la huella del árbol en diferentes tipos de bosque y época del año para las distintas variables estudiadas. Por ejemplo, en el caso del amonio, el efecto del árbol se restringió a los primeros metros desde el tronco en verano, sin embargo, se extendió más allá del dosel en primavera. Además, el efecto del árbol disminuyó más rápidamente con la distancia en los bosques cerrados que en los bosques abiertos. Por su parte, en el caso del nitrato, la disminución del efecto de los árboles con la distancia también fue más pronunciado en verano que en primavera, aunque a diferencia del amonio, la disminución del efecto del árbol con la distancia fue más acentuado en bosques cerrados que en bosques abiertos. Estos resultados ponen de manifiesto la complejidad del efecto de los árboles sobre la distribución espacial de variables clave de los ciclos biogeoquímicos en los bosques mediterráneos.

Variaciones temporales de los efectos del decaimiento de Q. suber en los procesos del suelo

Una de las principales aportaciones de esta tesis al estudio de las consecuencias del incremento de la mortalidad de árboles en los procesos ecosistémicos es que se realiza en ecosistemas mediterráneos, cuyo funcionamiento difiere en gran medida de los ecosistemas templados, donde hasta ahora se han centrado la mayoría de estudios en esta materia (Hicke *et al.*, 2012; Anderegg *et al.*, 2013). Una de las principales diferencias entre ecosistemas templados y mediterráneos es la fuerte variabilidad temporal, tanto intra- como inter-anual, en la disponibilidad de agua. Esta gran variabilidad podría

modular las consecuencias de la mortalidad de árboles en el funcionamiento del suelo, debido al papel que tiene el agua en el control de procesos como la descomposición o la respiración del suelo (Asensio *et al.*, 2007; Galmés *et al.*, 2007). En este sentido, los resultados del capítulo 3 resultan novedosos ya que el estudio se realizó en dos estaciones con diferencias contrastadas en cuanto a disponibilidad hídrica (primavera y verano) y durante tres años con marcadas diferencias en las precipitaciones (2010, 2011 y 2012). Nuestros resultados detectaron una baja variabilidad interanual en los efectos del estado de salud en los procesos del suelo, pero una elevada variabilidad intra-anual, detectándose principalmente los efectos durante el verano. Por tanto, este trabajo demuestra la importancia de considerar la variabilidad intra-anual en los estudios sobre las consecuencias del decaimiento en bosques mediterráneos para poder tener una visión completa de sus efectos en el funcionamiento del suelo.

Efectos interactivos entre el cambio climático y el decaimiento de *Q. suber*

Los bosques situados en la cuenca mediterránea tendrán que enfrentarse en los próximos años a incrementos de las temperaturas y descensos de la humedad del suelo asociados al cambio climático (Lindner *et al.*, 2010; Doblas-Miranda *et al.*, 2015). Cambios en la temperatura y la humedad del suelo podrían tener consecuencias sobre la mineralización de la materia orgánica y la respiración microbiana debido al papel que tienen ambas variables en el metabolismo microbiano (p. ej. Howard y Howard, 1993; MacDonald *et al.*, 1995). El incremento de la temperatura podría estimular la actividad microbiana y la descomposición de la materia orgánica, lo que podría tener un papel positivo en la mineralización de nutrientes, incrementando su disponibilidad (p. ej. Leirós *et al.*, 1999; Paul *et al.*, 2003; Szukics *et al.*, 2010). Sin embargo, al mismo tiempo, las altas temperaturas podrían conllevar un incremento de la inmovilización de nutrientes por los microrganismos del suelo, lo que dificultaría detectar estos efectos positivos de la temperatura (Niklińska *et al.*, 1999; Barrett y Burke, 2002). Por su parte, la disminución de la humedad del suelo podría tener consecuencias negativas para la actividad microbiana y la descomposición de la materia orgánica (Howard y Howard, 1993). Sin embargo, determinados grupos de organismos podrían continuar descomponiendo la materia orgánica incluso en condiciones de sequía, lo que evitaría detectar efectos negativos de la falta de humedad sobre los procesos del suelo (Matías *et al.*, 2011;

Auyeung *et al.*, 2013). Estos trabajos demuestran la complejidad para predecir la respuesta de los procesos del suelo ante los futuros escenarios de cambio climático.

En esta tesis se ha detectado que, en general, tanto la sequía como el incremento de las temperaturas predichos como consecuencias del cambio climático en la cuenca mediterránea podrían afectar tanto a la respiración del suelo como a la disponibilidad de nitrógeno y fósforo (capítulo 5). En general, se detectó un efecto negativo de la sequía y un efecto positivo del incremento de la temperatura sobre la respiración del suelo y la disponibilidad de nitrógeno. Sin embargo, fue la humedad del suelo el factor que provocó efectos de mayor magnitud en las variables estudiadas. El mayor impacto que tiene la sequía sobre los ciclos del carbono y nitrógeno podría ocultar el potencial efecto positivo de la temperatura sobre estas variables. Por tanto, este trabajo sugiere que los cambios predichos en la humedad del suelo podrían tener impactos más importantes que los cambios predichos en la temperatura en los ciclos de carbono, nitrógeno y fósforo. Esto podría estar influenciado por el hecho de que el agua es generalmente el factor limitante en los ecosistemas mediterráneos (Rey *et al.*, 2002; Emmett *et al.*, 2004), por lo que pequeños cambios en su disponibilidad se traducirían en importantes cambios funcionales del ecosistema.

Los estudios de efectos interactivos entre diferentes factores de cambio global son esenciales para poder comprender y predecir cómo los sistemas forestales funcionarán en un futuro cercano. Como se comentó anteriormente, además del efecto de los patógenos invasores, los bosques mediterráneos también se verán afectados por el incremento de la aridez debido al cambio climático. Sin embargo, el estudio de estos efectos por separado no nos permiten predecir correctamente cuál sería su efecto actuando simultáneamente (e.g. Luo *et al.*, 2008; Morillas *et al.*, 2015). En el capítulo 5 se demostró el papel tan importante que el patógeno invasor *P. cinnamomi* puede tener como modificador de la respuesta del funcionamiento del bosque ante factores de cambio climático, tal y como se predijo en la primera hipótesis del capítulo 5. Los resultados mostraron que la defoliación y mortalidad provocada por el patógeno se podrían traducir en unos efectos en la respiración y la disponibilidad de nitrógeno de mayor magnitud que lo que se podría esperar si ocurrieran en ausencia del patógeno. Estos efectos interactivos sinérgicos encontrados entre la sequía y la presencia de árboles afectados por el patógeno pone en evidencia la necesidad de considerar los efectos interactivos cuando se estudian diferentes factores de cambio global (Dieleman *et al.*, 2012; Zhou *et al.*, 2016).

Cuando se comparó la respuesta de la respiración del suelo y la disponibilidad de nutrientes ante futuros escenarios de calentamiento y sequía para 2050 y 2100 en suelos bajo árboles sanos y decaimiento, se observaron importantes diferencias en cuanto a la resistencia al cambio de estos procesos. A pesar de no observarse diferencias en la cantidad y diversidad de las comunidades microbianas bajo árboles con diferente estado de salud (capítulo 4), los resultados demuestran que el funcionamiento de las comunidades microbianas difiere cuando se someten a las condiciones de temperatura y humedad del suelo predichas en los próximos años como consecuencia del cambio climático. Nuestros resultados sugieren que, aunque no haya cambios en la diversidad (al menos de grupos funcionales) de la comunidad microbiana, la resistencia al cambio de las comunidades asociadas a árboles sanos es mayor que la de las comunidades microbianas asociadas a árboles defoliados o muertos. Esto probablemente es debido a que las comunidades microbianas bajo árboles sanos mantendrían determinados grupos funcionales tolerantes a condiciones típicas de aridez que ocurren en estos sistemas durante el verano (Barnard *et al.*, 2013; Curiel Yuste *et al.*, 2014), y que no se encuentren bajo árboles defoliados o muertos.

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Los resultados presentados en esta tesis permiten ampliar el conocimiento sobre las consecuencias del aumento de la mortalidad de árboles en los ciclos biogeoquímicos en bosques mediterráneos. Se ha demostrado cómo los árboles infectados no parecen poder adaptar su fisiología (eficiencia del uso del agua y crecimiento secundario) a la pérdida de raíces finas, de manera que su respuesta al estrés hídrico resultante sería la defoliación y eventual muerte del individuo. La defoliación y muerte de los individuos de *Q. suber* provocaron importantes consecuencias tanto para la mineralización de la materia orgánica, como para la disponibilidad de nutrientes tan importantes como el nitrógeno y fósforo. No solo la defoliación y muerte de *Q. suber* podría modificar los procesos del suelo, sino que, además, esta tesis doctoral ha mostrado como los cambios en la abundancia relativa de la composición de especies podrían desencadenar importantes cambios en el funcionamiento del sistema, dependiendo de las características de las especies que sustituyan al *Q. suber* a lo largo del proceso de sucesión secundaria. Finalmente, los cambios provocados en el suelo bajo árboles afectados, provocará que los

procesos del suelo sean menos resistentes a los futuros escenarios predichos por el cambio climático, lo que determinará el funcionamiento del ecosistema en las próximas décadas.

En la península ibérica, la conservación de los ecosistemas forestales dominados por especies de *Quercus* perennifolios, entre los que se encuentran las valiosas dehesas y montados, está fuertemente amenazada dada las altas tasas de mortalidad del arbolado adulto y la falta de regeneración (Ibáñez *et al.*, 2015). Estos sistemas proporcionan unos servicios de enorme importancia para la región tanto a nivel ecológico como económico y social, como la provisión de corcho y madera, forraje para el ganado, hábitats para animales, protección del suelo, secuestro de carbono, reducción del riesgo de inundaciones, etc. Además del problema económico que supone la pérdida del arbolado, esta tesis doctoral ha mostrado cómo, una vez los individuos de *Q. suber* son afectados y mueren, se producen importantes cambios en el funcionamiento del bosque lo que, a su vez, tendrá importantes consecuencias para los servicios ecosistémicos que nos aporta. A su vez, estos impactos negativos podrían incrementarse en las próximas décadas debido al posible incremento tanto en la actividad como en la distribución de *P. cinnamomi* predicho en distintas regiones del mundo como consecuencia de escenarios futuros caracterizados por un clima más cálido (Burgess *et al.*, 2017). Un conocimiento más profundo sobre el funcionamiento de bosques afectados por *P. cinnamomi* resulta esencial para poder desarrollar una gestión sostenible y adaptativa basada en el equilibrio del ecosistema, lo que permitirá su conservación a largo plazo.

Referencias

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim JH, Allard G, Running SW, Semerci A, Cobb N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* 259(4): 660-684.

Allison FE 1973. Nonbiological Immobilization of Nitrogen. Developments in Soil Science, Volume 3. Soil Organic Matter and its Role in Crop Production. Amsterdam: Elsevier, 206-229.

Allison SD, Martiny JBH. 2008. Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences* 105(Supplement 1): 11512-11519.

Anderegg WRL, Hicke JA, Fisher RA, Allen CD, Aukema J, Bentz B, Hood S, Lichstein JW, Macalady AK, McDowell N, Pan Y, Raffa K, Sala A, Shaw JD, Stephenson NL, Tague C, Zeppel M. 2015. Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist* 208(3): 674-683.

Anderegg WRL, Kane JM, Anderegg LDL. 2013. Consequences of widespread tree mortality triggered by drought and temperature stress. *Nature Climate Change* 3(1): 30-36.

Asensio D, Peñuelas J, Ogaya R, Llusià J. 2007. Seasonal soil and leaf CO_2 exchange rates in a Mediterranean holm oak forest and their responses to drought conditions. *Atmospheric Environment* 41(11): 2447-2455.

Auyeung DSN, Suseela V, Dukes JS. 2013. Warming and drought reduce temperature sensitivity of nitrogen transformations. *Global Change Biology* 19(2): 662-676.

Barnard RL, Osborne CA, Firestone MK. 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. *ISME J* 7(11): 2229-2241.

Barrett JE, Burke IC. 2002. Nitrogen Retention in Semiarid Ecosystems Across a Soil Organic-Matter Gradient. *Ecological Applications* 12(3): 878-890.

Bell T, Gessner MO, Griffiths RI, McLaren JR, Morin PJ, van der Heijden M, van der Putten W. 2009. Microbial biodiversity and ecosystem functioning under controlled conditions and in the wild. *Biodiversity, Ecosystem Functioning, and Human Wellbeing: An Ecological and Economic Perspective. Oxford University Press: Oxford*: 121-133.

Burgess TI, Scott JK, Mcdougall KL, Stukely MJC, Crane C, Dunstan WA, Brigg F, Andjic V, White D, Rudman T, Arentz F, Ota N, Hardy GESJ. 2017. Current and projected global distribution of Phytophthora cinnamomi, one of the world's worst plant pathogens. *Global Change Biology* 23(4): 1661-1674.

Canham CD, Uriarte M. 2006. Analysis of neighborhood dynamics of forest ecosystems using likelihood methods and modeling. *Ecological Applications* 16(1): 62-73.

Carnicer J, Coll M, Ninyerola M, Pons X, Sánchez G, Peñuelas J. 2011. Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* 108(4): 1474-1478.

Clow DW, Rhoades C, Briggs J, Caldwell M, Lewis WM. 2011. Responses of soil and water chemistry to mountain pine beetle induced tree mortality in Grand County, Colorado, USA. *Applied Geochemistry* 26(SUPPL.): S174-S178.

Cobb RC, Eviner VT, Rizzo DM. 2013. Mortality and community changes drive sudden oak death impacts on litterfall and soil nitrogen cycling. *New Phytologist* 200(2): 422-431.

Corcobado T, Cubera E, Moreno G, Solla A. 2013. *Quercus ilex* forests are influenced by annual variations in water table, soil water deficit and fine root loss caused by *Phytophthora cinnamomi*. *Agricultural and Forest Meteorology* 169: 92-99.

Curiel Yuste J, Fernandez-Gonzalez A, Fernandez-Lopez M, Ogaya R, Peñuelas J, Sardans J, Lloret F. 2014. Strong functional stability of soil microbial communities under semiarid Mediterranean conditions and subjected to long-term shifts in baseline precipitation. *Soil Biology and Biochemistry* 69: 223-233.

Delgado-Baquerizo M, Giaramida L, Reich PB, Khachane AN, Hamonts K, Edwards C, Lawton LA, Singh BK, Brophy C. 2016. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. *Journal of Ecology* 104(4): 936-946.

Dieleman WIJ, Vicca S, Dijkstra FA, Hagedorn F, Hovenden MJ, Larsen KS, Morgan JA, Volder A, Beier C, Dukes JS, King J, Leuzinger S, Linder S, Luo Y, Oren R, De Angelis P, Tingey D, Hoosbeek MR, Janssens IA. 2012. Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO2 and temperature. *Global Change Biology* 18(9): 2681-2693.

Doblas-Miranda E, Martínez-Vilalta J, Lloret F, Álvarez A, Ávila A, Bonet F, Brotons L, Castro J, Curiel Yuste J, Díaz M. 2015. Reassessing global change research priorities in mediterranean terrestrial ecosystems: how far have we come and where do we go from here? *Global Ecology and Biogeography* 24(1): 25-43.

Edburg SL, Hicke JA, Lawrence DM, Thornton PE. 2011. Simulating coupled carbon and nitrogen dynamics following mountain pine beetle outbreaks in the western United States. *Journal of Geophysical Research G: Biogeosciences* 116(4): G04033.

Emmett BA, Beier C, Estiarte M, Tietema A, Kristensen HL, Williams D, Peñuelas J, Schmidt I, Sowerby A. 2004. The response of soil processes to climate change:

Results from manipulation studies of shrublands across an environmental gradient. *Ecosystems* 7(6): 625-637.

Finzi AC, Austin AT, Cleland EE, Frey SD, Houlton BZ, Wallenstein MD. 2011. Responses and feedbacks of coupled biogeochemical cycles to climate change: examples from terrestrial ecosystems. *Frontiers in Ecology and the Environment* 9(1): 61-67.

Flower CE, Knight KS, Gonzalez-Meler MA. 2013. Impacts of the emerald ash borer (Agrilus planipennis Fairmaire) induced ash (Fraxinus spp.) mortality on forest carbon cycling and successional dynamics in the eastern United States. *Biological Invasions* 15(4): 931-944.

Galmés J, Medrano H, Flexas J. 2007. Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytologist* 175(1): 81-93.

Gómez-Aparicio L, Canham CD. 2008. Neighborhood models of the effects of invasive tree species on ecosystem processes. *Ecological Monographs* 78(1): 69-86.

Hassink J. 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. *Plant and Soil* 191(1): 77-87.

Hassink J, Whitmore AP. 1997. A model of the physical protection of organic matter in soils. *Soil Science Society of America Journal* 61(1): 131-139.

Hicke JA, Allen CD, Desai AR, Dietze MC, Hall RJ, Hogg EH, Kashian DM, Moore D, Raffa KF, Sturrock RN, Vogelmann J. 2012. Effects of biotic disturbances on forest carbon cycling in the United States and Canada. *Global Change Biology* 18(1): 7-34.

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Hogberg MN, Nyberg G, Ottosson-Lofvenius M, Read DJ. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* 411(6839): 789-792.

Högberg P, Read DJ. 2006. Towards a more plant physiological perspective on soil ecology. *Trends in Ecology & Evolution* 21(10): 548-554.

Howard DM, Howard PJA. 1993. Relationships between CO_2 evolution, moisture content and temperature for a range of soil types. *Soil Biology & Biochemistry* 25(11): 1537-1546.

Ibáñez B, Gómez-Aparicio L, Ávila JM, Pérez-Ramos IM, Marañón T. 2017. Effects of Quercus suber Decline on Woody Plant Regeneration: Potential Implications for Successional Dynamics in Mediterranean Forests. *Ecosystems* 20(3): 630-644.

Ibáñez B, Gómez-Aparicio L, Stoll P, Ávila JM, Pérez-Ramos IM, Marañón T. 2015. A neighborhood analysis of the consequences of *Quercus suber* decline for regeneration dynamics in Mediterranean forests. *PloS one* 10(2): e0117827.

Kizlinski ML, Orwig DA, Cobb RC, Foster DR. 2002. Direct and indirect ecosystem consequences of an invasive pest on forests dominated by eastern hemlock. *Journal of Biogeography* 29(10-11): 1489-1503.

Leirós MC, Trasar-Cepeda C, Seoane S, Gil-Sotres F. 1999. Dependence of mineralization of soil organic matter on temperature and moisture. *Soil Biology and Biochemistry* 31(3): 327-335.

Levy-Varon JH, Schuster WS, Griffin KL. 2012. The autotrophic contribution to soil respiration in a northern temperate deciduous forest and its response to stand disturbance. *Oecologia* 169(1): 211-220.

Levy-Varon JH, Schuster WSF, Griffin KL. 2014. Rapid rebound of soil respiration following partial stand disturbance by tree girdling in a temperate deciduous forest. *Oecologia* 174(4): 1415-1424.

Linares JC, Camarero JJ. 2012. From pattern to process: linking intrinsic water-use efficiency to drought-induced forest decline. *Global Change Biology* 18(3): 1000-1015.

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M. 2010. Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecology and Management* 259(4): 698-709.

Lovett GM, Arthur MA, Weathers KC, Griffin JM. 2010. Long-term changes in forest carbon and nitrogen cycling caused by an introduced pest/pathogen complex. *Ecosystems* 13(8): 1188-1200.

Ludwig F, de Kroon H, Berendse F, Prins HH. 2004. The influence of savanna trees on nutrient, water and light availability and the understorey vegetation. *Plant Ecology* 170(1): 93-105.

Luo Y, Gerten D, Le Maire G, Parton WJ, Weng E, Zhou X, Keough C, Beier C, Ciais P, Cramer W, Dukes JS, Emmett B, Hanson PJ, Knapp A, Linder S, Nepstad DAN, Rustad L. 2008. Modeled interactive effects of precipitation, temperature, and [CO2] on ecosystem carbon and water dynamics in different climatic zones. *Global Change Biology* 14(9): 1986-1999.

Luque J, Cohen M, Save R, Biel C, Alvarez IF. 1999. Effects of three fungal pathogens on water relations, chlorophyll fluorescence and growth of *Quercus suber* L. *Annals of Forest Science* 56(1): 19-26.

MacDonald NW, Zak DR, Pregitzer KS. 1995. Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralization. *Soil Science Society of America Journal* 59(1): 233-240.

Matías L, Castro J, Zamora R. 2011. Soil-nutrient availability under a global-change scenario in a Mediterranean mountain ecosystem. *Global Change Biology* 17(4): 1646-1657.

Maurel M, Robin C, Capron G, Desprez-Loustau ML. 2001. Effects of root damage associated with *Phytophthora cinnamomi* on water relations, biomass accumulation, mineral nutrition and vulnerability to water deficit of five oak and chestnut species. *Forest Pathology* 31(6): 353-369.

Moreira AC, Martins JMS. 2005. Influence of site factors on the impact of Phytophthora cinnamomi in cork oak stands in Portugal. *Forest Pathology* 35(3): 145-162.

Morillas L, Duran J, Rodriguez A, Roales J, Gallardo A, Lovett GM, Groffman PM. 2015. Nitrogen supply modulates the effect of changes in drying-rewetting frequency on soil C and N cycling and greenhouse gas exchange. *Global Change Biology* 21(10): 3854-3863.

Müller T, Höper H. 2004. Soil organic matter turnover as a function of the soil clay content: consequences for model applications. *Soil Biology and Biochemistry* 36(6): 877-888.

Niklińska M, Maryański M, Laskowski R. 1999. Effect of temperature on humus respiration rate and nitrogen mineralization: implications for global climate change. *Biogeochemistry* 44(3): 239-257.

Nuckolls AE, Wurzburger N, Ford CR, Hendrick RL, Vose JM, Kloeppel BD. 2009. Hemlock declines rapidly with hemlock woolly adelgid infestation: impacts on the carbon cycle of Southern Appalachian forests. *Ecosystems* 12(2): 179-190.

Paul KI, Polglase PJ, O'Connell AM, Carlyle JC, Smethurst PJ, Khanna PK. 2003. Defining the relation between soil water content and net nitrogen mineralization. *European Journal of Soil Science* 54(1): 39-47.

Rey A, Pegoraro E, Tedeschi V, De Parri I, Jarvis PG, Valentini R. 2002. Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. *Global Change Biology* 8(9): 851-866.

Robin C, Capron G, Desprez-Loustau ML. 2001. Root infection by *Phytophthora cinnamomi* in seedlings of three oak species. *Plant Pathology* 50(6): 708-716.

Sangüesa-Barreda G, Linares JC, Julio Camarero J. 2013. Drought and mistletoe reduce growth and water-use efficiency of Scots pine. *Forest Ecology and Management* 296(0): 64-73.

Schneider K, Turrión MB, Grierson PF, Gallardo JF. 2001. Phosphatase activity, microbial phosphorus, and fine root growth in forest soils in the Sierra de Gata, western central Spain. *Biology and Fertility of Soils* 34(3): 151-155.

Sghaier-Hammami B, Valero-Galvan J, Romero-Rodriguez MC, Navarro-Cerrillo RM, Abdelly C, Jorrin-Novo J. 2013. Physiological and proteomics analyses of Holm oak (*Quercus ilex* subsp *ballota* Desf. Samp.) responses to Phytophthora cinnamomi. *Plant Physiology and Biochemistry* 71: 191-202.

Søe AR, Buchmann N. 2005. Spatial and temporal variations in soil respiration in relation to stand structure and soil parameters in an unmanaged beech forest. *Tree Physiology* 25(11): 1427-1436.

Szukics U, Abell GCJ, Hödl V, Mitter B, Sessitsch A, Hackl E, Zechmeister-Boltenstern S. 2010. Nitrifiers and denitrifiers respond rapidly to changed moisture and increasing temperature in a pristine forest soil. *FEMS Microbiology Ecology* 72(3): 395-406.

Trumbore S, Brando P, Hartmann H. 2015. Forest health and global change. *Science* 349(6250): 814-818.

van Mantgem PJ, Stephenson NL, Byrne JC, Daniels LD, Franklin JF, Fule PZ, Harmon ME, Larson AJ, Smith JM, Taylor AH, Veblen TT. 2009. Widespread increase of tree mortality rates in the western United States. *Science* 323(5913): 521-524.

Van Veen JA, Kuikman PJ. 1990. Soil structural aspects of decomposition of organic matter by micro-organisms. *Biogeochemistry* 11(3): 213-233.

Wang WF, Peng CH, Kneeshaw DD, Larocque GR, Luo ZB. 2012. Drought-induced tree mortality: ecological consequences, causes, and modeling. *Environmental Reviews* 20(2): 109-121.

Xiong Y, D'Atri JJ, Fu S, Xia H, Seastedt TR. 2011. Rapid soil organic matter loss from forest dieback in a subalpine coniferous ecosystem. *Soil Biology and Biochemistry* 43(12): 2450-2456.

Zhang B, Zhou X, Zhou L, Ju R. 2015. A global synthesis of below-ground carbon responses to biotic disturbance: a meta-analysis. *Global Ecology and Biogeography* 24(2): 126-138.

Zhou L, Zhou X, Shao J, Nie Y, He Y, Jiang L, Wu Z, Hosseini Bai S. 2016. Interactive effects of global change factors on soil respiration and its components: a meta-analysis. *Global Change Biology* 22(9): 3157-3169.

CAPÍTULO 7

CONCLUSIONES GENERALES

CONCLUSIONES

1. En esta tesis doctoral se han mostrado las altas tasas de mortalidad de *Quercus suber* que pueden alcanzarse en bosques mixtos mediterráneos afectados por el patógeno exótico *Phytophthora cinnamomi*. A escala individual no se detectaron diferencias significativas en la eficiencia en el uso del agua ni en el crecimiento secundario entre árboles defoliados y sanos. Sin embargo, a escala local y regional, la eficiencia del uso del agua de árboles de *Q. suber* aumentó bajo condiciones de estrés como la sequía del suelo o alta abundancia de *P. cinnamomi*.

2. Los resultados de esta tesis mostraron que, aunque *Q. suber* manifestó una respuesta ecofisiológica al estrés provocada por la escasez de agua a escala local y regional, la falta de una clara relación entre el estado de salud y la eficiencia del uso del agua y crecimiento secundario parecen demostrar una pobre respuesta adaptativa de *Q. suber* al ataque del patógeno *P. cinnamomi*.

3. La defoliación y muerte de individuos de *Q. suber* se tradujo en importantes reducciones en las tasas de respiración del suelo y la disponibilidad de fósforo, probablemente como consecuencia de la reducción en la actividad de las raíces asociada al decaimiento de *Q. suber*. El signo del efecto del estado de salud de *Q. suber* en la disponibilidad de nitrógeno varió entre tipos de bosque, debido posiblemente a diferencias en el microclima o la textura del suelo. Los modelos de vecindad sugieren que los árboles más grandes pueden tener unos impactos desproporcionados sobre la respiración del suelo y la disponibilidad de nutrientes, lo que confiere al proceso de decaimiento una elevada capacidad de modificar los ciclos biogeoquímicos.

4. Los efectos del decaimiento de *Q. suber* en los ciclos biogeoquímicos mostraron una baja variabilidad inter-anual pero elevada variabilidad intra-anual. Dichos efectos fueron detectados principalmente durante verano, lo que muestra la importancia de incluir la componente temporal para conocer el impacto del decaimiento en ecosistemas mediterráneos.

5. El decaimiento de *Q. suber* se tradujo en cambios sustanciales en los ciclos de carbono y nitrógeno, pero no tuvo efectos en la comunidad microbiana en términos de diversidad funcional o biomasa microbiana.

6. El efecto de la mortalidad asociada al patógeno varió en signo y magnitud entre bosques con diferentes características del suelo, particularmente la textura. El alto contenido en arcilla típico de los bosques abiertos de *Q. suber* y *O. europaea* podría proteger a la materia orgánica frente a la descomposición y modificar el impacto del decaimiento *Q. suber* en las dinámicas de la materia orgánica.

7. El remplazamiento de *Q. suber* por *O. europaea* podría provocar un descenso en nitrógeno y fósforo, mientras que una sustitución por *Q. canariensis* podría producir suelos más fértiles con mayores contenidos en carbono orgánico y nitrógeno inorgánico. Estos resultados sugieren que los efectos indirectos de la mortalidad de una especie arbórea dependerán de la identidad de la especie que lo sustituya, resultando en diferentes implicaciones en el ecosistema a largo plazo, lo que puede llevar a una aceleración o deceleración de los ciclos de nutrientes.

8. El decaimiento de *Q. suber* y el cambio climático podrían interaccionar para alterar los ciclos biogeoquímicos en bosques invadidos por *P. cinnamomi*, con un impacto mucho mayor al que tendrían si estos factores actuasen de forma independiente. El descenso predicho en la humedad del suelo conllevó una reducción en la respiración del suelo y la disponibilidad de nitrógeno, pero la magnitud de este descenso varió en función de la salud del árbol. Los modelos mostraron un efecto negativo aditivo del decaimiento de *Q. suber*, el calentamiento y la sequía en la disponibilidad de fósforo.

9. El decaimiento de *Q. suber* podría contribuir a la alteración de los ciclos biogeoquímicos en los ecosistemas mediterráneos en las próximas décadas, haciendo a los suelos menos resistentes al incremento de la temperatura y la sequía predichos por los distintos escenarios de cambio climático.

10. Los resultados de esta tesis doctoral muestran que la mortalidad arbórea asociada a patógenos exóticos conlleva importantes cambios en los ciclos biogeoquímicos de bosques mixtos mediterráneos. Debido al incremento tanto en la actividad como en la

distribución de *P. cinnamomi* predicho en escenarios futuros caracterizados por un clima más cálido, podrían esperarse cambios severos en los balances de carbono y nutrientes en bosques de todo el mundo invadidos o bajo alto riesgo de invasión por este agresivo patógeno.

CONCLUSIONS

1. This doctoral thesis has showed the high mortality rates of *Quercus suber* trees that can be detected in Mediterranean mixed forests greatly affected by the invasive pathogen *Phytophthora cinnamomi*. Significant differences were not observed between defoliated and healthy trees, either in terms of water use efficiency or growth at the whole-tree scale. However, we found that limiting conditions, such as low soil depth and high pathogen abundance, induced trees to higher water use efficiency at local and landscape scales.

2. The results of this thesis showed that, although *Q. suber* was able to respond ecophysiologically to water stress at local and landscape scale, the lack of a straightforward relationship between the health status of trees and water use efficiency and secondary growth seems to demonstrate a poor adaptive response of *Q. suber* to the attack of *P. cinnamomi.*

3. The defoliation and death of *Q. suber* trees translated into important reductions in soil respiration rates and phosphorus availability, probably as a consequence of the reduction in root activity associated with *Q. suber* decline. The sign of the effect of the health status of *Q. suber* on nitrogen availability varied among forest types, probably due to differences in microclimate or soil texture. The neighborhood models suggest that large trees might have disproportionate impacts on soil respiration and nutrient availability, conferring to the process of forest dieback an extremely high capacity to modify biogeochemical cycles.

4. The effects of *Q. suber* decline on biogeochemical cycles experienced low inter-annual but high intra-annual variability. The effects of the defoliation and death of *Q. suber* were observed mainly in summer. This finding evidences the importance of considering the temporal component of these processes to understand the impacts of *Q. suber* decline in Mediterranean systems.

5. *Q. suber* decline translated into substantial modifications of the carbon and nitrogen cycles, but with no effects on the microbial community in terms of functional diversity or microbial biomass.

6. The effect of pathogen-induced mortality on carbon and nitrogen differed in sign and magnitude between forests with contrasted soil characteristics, particularly texture. The high clay content that characterized the open woodlands of *Q. suber* and *O. europaea* might protect the organic matter against decomposition and might alter the impact of *Q. suber* decline on the dynamics of soil organic matter.

7. A potential substitution of *Q. suber* by *O. europaea* might result in soils impoverished in N and P, whereas a replacement by *Q. canariensis* could lead to more fertile soils with higher content of soil organic carbon and inorganic nitrogen. These findings suggest that the indirect effects of the mortality of a given tree species will be strongly dependent on the identity of the replacement species, resulting in different long-term ecosystem implications that can vary from acceleration to deceleration of nutrient cycles.

8. *Q. suber* decline and climate change might interact to alter biogeochemical cycles in forests affected by *P. cinnamomi* to a much larger extent than predicted when considered separately. Predicted drought translated into a reduction in soil respiration and nitrogen availability, but the magnitude of such decrease varied strongly depending on the tree health. Models showed negative additive effect of *Q. suber* decline, warming and drought on phosphorus availability.

9. *Q. suber* decline may contribute to the alteration of biogeochemical cycles in Mediterranean ecosystems in the future, making their soils less resistant to the predicted warming and drought under the ongoing climatic change.

10. This doctoral thesis demonstrates that pathogen-induced tree mortality leads to important alterations in biogeochemical cycles of Mediterranean mixed forests. Since the distribution and activity of *P. cinnamomi* is expected to increase in future warmed scenarios, we can expect profound impacts on carbon and nutrient balances in forests worldwide where this aggressive pathogen is already stablished or have a high risk of invasion.

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